# Plasticity of the Mauthner-cell associated C-start circuitry

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Für meine Eltern und meine Freundin

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# ZUSAMMENFASSUNG

Das Mauthner (M)-Zellen assoziierte retikulärspinale Netzwerk von Fischen und Kaulquappen erlangte im Wesentlichen aufgrund seiner Zugänglichkeit auf zellulärer Ebene und durch die enorme Größe der zwei M-Zellen Bedeutung in der Neurowissenschaft. Trotz der erstaunlicherweise geringen Größe dieses neuronalen Netzwerks mit nur einigen hundert Zellen und wenigen synaptischen Verbindungen, die von einem Individuum zum nächsten identifiziert werden können, ist es für ein wichtiges Manöver verantwortlich, den Flucht C-start der meisten Knochenfische. Diese Studie trägt wichtige Aspekte zu der Auffassung bei, dass das Flucht-Netzwerk, obwohl klein, hoch anspruchsvolle und plastische C-Start Entscheidungen steuert. In Schützenfischen lenkt es den sogenannten prädiktiven C-Start: Anhand der visuellen Beurteilung der Anfangsbewegung von abgeschossenen, in der Luft frei fallenden Futterobjekten, führen diese Fische einen C-Start aus, der sie perfekt ausgerichtet auf den späteren Auftreffpunkt dreht und sie mit angepasster Geschwindigkeit abstößt, um die Beute zum richtigen Zeitpunkt zu fangen. Die hohe Präzision in Bezug auf den Zielpunkt und die vollständige experimentelle Kontrolle der prädiktiven C-Starts machen diese Entscheidungen zu wertvollen Modellsystemen dafür, wie im Nervensystem Entscheidungen getroffen werden und wie Umwelteinflüsse die zugrundeliegenden Netzwerke beeinflussen. Zusammen mit der Zugänglichkeit der Netzwerke eignen sich diese Starts hervorragend für eine Untersuchung des Ausmaßes der neuronalen Plastizität und der Aufdeckung innewohnender Puffermechanismen für das Erreichen funktioneller Stabilität. Hier lege ich vier zentrale Ergebnisse vor.

(1) Funktionale Stabilität. Temperatur beeinflusst die Physiologie poikilothermer Tiere und daher die Eigenschaften ihrer neuronalen Netzwerke, was ihre Leistung beeinträchtigen könnte. Dennoch müssen Schützenfische die richtigen Entscheidungen treffen: die Wahl eines ungeeigneten Drehwinkels oder Startgeschwindigkeit bedeutet den Verlust der Beute an die zahlreichen Konkurrenten. Das Entscheidungs-Netzwerk sollte daher gepuffert werden, so dass die Funktion nicht durch Temperaturveränderungen beeinflusst wird. Tatsächlich konnte ich herausfinden, dass nach einer kurzen Akklimatisierungsphase die Funktion der prädiktiven C-Starts der Schützenfische in einem Temperaturbereich vollständig erhalten blieb. Bei allen Temperaturen wurde die Reaktionsfähigkeit aufrechterhalten, und die Fische wählten genaue Drehungen im gesamten Winkelbereich. Darüber hinaus passten sie mit gleicher Genauigkeit bei allen Temperaturen ihre Startgeschwindigkeit an die verbleibende Distanz zum Auftreffpunkt ihrer Beute und der verbleibenden Zeit bis zum Aufschlag an. Andere Aspekte wie Latenz oder die C-Start Kinematik waren indes nicht oder nur teilweise Temperatur kompensiert.

(2) Intrinsische Qualitätssicherung. Verringern der Helligkeit reduziert die Zuverlässig-

keit der visuellen Information, auf dessen Grundlage die prädiktiven Starts ausgeführt werden. Basierend auf typischen Befunden vieler Entscheidungen sollte dies zu einem Zerfall der Genauigkeit führen. Jedoch konnte ich herausfinden, dass dies nicht für die Schützenfisch C-Starts zutrifft. Die Schützenfische entschieden sich richtig im gesamten Bereich von Eingangskonstellationen mit konstanter Genauigkeit bis zu einer reduzierten Helligkeit von $5\times10^{-3}$ cd m$^{-2}$. Unterhalb dieser Schwelle wurden keine prädiktiven C-Starts ausgelöst, obwohl die Fische bei dieser und weit aus geringeren Lichtverhältnissen nachweislich sehen konnten und präzise auf Beute schossen. Trotz der Verringerung der Auslösewahrscheinlichkeit und gleichzeitigem Anstieg der Latenz (um die Beute-Anfangsbewegung verlängert zu evaluieren), blieben die grundlegenden kinematischen Eigenschaften der C-Starts im Dunklen unverändert. Meine Ergebnisse weisen auf einen Kontrollmechanismus noch unbekannter Natur hin, der sicherstellt, dass C-Starts nur dann ausgelöst werden, wenn sie erwartungsgemäß exakt auf den späteren Auftreffpunkt gerichtet sind.

(3) Das Netzwerk wird auch in anderen Arten ähnlich genutzt. Da der Großteil der Knochenfische das M-Zellen assoziierte Netzwerk besitzt, kam die Frage auf, ob andere Arten neben dem Schützenfisch dieses Netzwerk nicht nur für die Flucht, sondern auch für genau abgestimmte Starts in ihrer Ernährungsweise benutzen. Anhand von Feldforschung in Costa Rica stellte ich fest, dass der Früchte fressende, in Flüssen lebende Machaca, *Brycon guatemalensis*, C-Starts in der Art und Weise wie Schützenfische verwendet. Wartend unterhalb von Bäumen mit reifen Feigen, frontal zur Strömung ausgerichtet, reagieren diese Fische auf die visuelle Information fallender Früchte bevor diese auf die Wasseroberfläche aufschlagen und starten in Richtung des späteren Auftreffpunkts. Der interessante Aspekt dieser Starts ist, dass die Fische (aber nicht ihr Zielobjekt) starker Strömung ausgesetzt sind. Erstaunlicherweise lösten die Fische dieses Problem gleich bei ihrem Start, indem sie sich in eine Richtung drehten, die optimal an die Abdrift auf ihrem Weg zum Ziel angepasst war.

(4) Das Netzwerk kann in adulten Zebrafischen neu programmiert werden. Es stellte sich die Frage, ob man noch einen Schritt weiter gehen könnte und das M-Zellen Netzwerk sogar durch zuvor Entscheidungs-irrelevante Information angepasst werden kann. Nachdem adulte Zebrafische ausschließlich einer Umwelt ausgesetzt wurden, in der das gesamte Futter auf ballistischen Flugbahnen aus der Luft fiel, konnten wir zeigen, dass das Netzwerk tatsächlich die Fallgesetze verinnerlicht hatte. Die Zebrafische waren imstande, genau abgestimmte C-Starts für beliebige Flugbahnen auszuwählen, sogar für zuvor völlig unbekannte. Daraus ergibt sich die einmalige Möglichkeit, den Aufbau der Repräsentation eines Physikgesetzes in einem kleinen definierten Netzwerk zu erforschen, und um zu verstehen, wie Gesetze der äußeren Welt in der Sprache der neuronalen Netzwerke dargestellt werden.

# SUMMARY

The Mauthner (M)-cell associated reticulospinal circuitry of fish and tadpoles has been important in neuroscience largely due to its accessibility on a cellular level and due to the enormous size of the two M-cells. Although this neuronal network is surprisingly small with a few hundred defined cells and few synaptic levels that can be identified from one individual to the next, it is responsible for an important manoeuvre, the escape C-start shown by most teleost fish. This study contributes several key issues to argue that the escape circuitry, although small, drives highly sophisticated and plastic C-start decisions. In archerfish it drives their so-called predictive C-start: Based on the visual sampling of the initial motion of dislodged aerial falling food objects, these fish initiate a C-start that turns them to be perfectly aligned to the later point of impact long before the food hits the water surface and pushes them off with a matched speed to make the catch just right in time. The high accuracy with its aim well-known to the inferred target point and the full experimental controllability of the predictive C-starts make these fast-start decisions valuable models of decision-making and of how environmental perturbations affect the underlying circuits. Given the accessibility of the neuronal networks, these starts are perfectly suited to assay the degree of the circuits' plasticity and reveal inherent buffering mechanisms to achieve functional stability.

(1) Functional stability. Temperature affects the physiology of poikilothermic animals and, thus, properties of their neuronal circuitry that could compromise performance. Yet, archerfish have to decide correctly: Selecting an inappropriate turn angle or take-off speed means loosing prey to the numerous competitors. The decision-network should thus be buffered so that function is not affected by changes in temperature. Indeed, I found that after a brief acclimation period the function of the archerfish predictive C-starts was fully maintained over a range of operating temperatures. At all temperatures full responsiveness was sustained and the fish selected accurate turns over the full angular range. Furthermore, the fish matched their take-off speed attained immediately after the end of the C-start to remaining distance to the prey's future landing point and remaining time with equal precision at all temperatures. Other aspects, however, such as latency and kinematics of the C-start are not or only partly temperature compensated.

(2) Intrinsic quality control. Dimming light reduces the reliability of the visual input the predictive starts are based on. Based on typical findings in many other decisions this

should lead to a decay in accuracy. However, I discovered that this does not hold for the archerfish C-start decisions. Archerfish decided correctly over the full range of input constellations with equal precision down to light levels of $5\times10^{-3}$ cd m$^{-2}$. Below, no more predictive C-starts were released – although the fish demonstrably were fully capable of seeing and accurately shooting at prey at this and even far lower light levels. While release probability decreased and latency increased to allow prolonged sampling of the prey's initial motion, the basic kinematic properties of C-starts were, however, unchanged under dim light conditions. My findings suggest an efficient pre-release control mechanism of yet unknown identity that ensures that C-starts are only released when they are expected to be accurate to the later point of catch.

(3) Similar use of the network in other species. Because the majority of teleost fish have M-cell associated circuitry we wondered whether other species might by able to use them like archerfish, not only to drive escapes but also to drive fine-tuned starts in feeding. In fieldwork, carried out in Costa Rica, I discovered that the fruit-eating riverine machaca, *Brycon guatemalensis*, also employs C-starts in an archerfish-like manner. Waiting underneath trees with ripe figs, head on against the stream, these fish respond to visual motion of falling fruits prior to their impact on the water surface and start right away to the later point of catch. The interesting aspect of these starts is that the fish (but not their target) encountered severe relative drift on their way. Surprisingly, the fish solved this problem right by their starts – rotating them on a direction that was already optimally adapted to the drift they would later encounter.

(4) The circuitry can be tuned in adult zebrafish. The previous finding triggered the question, if one could go one step further and test if the M-cell circuitry even can be tuned to previously decision-irrelevant information. After extensively exposing adult zebrafish to an environment in which all food was aerial and fell on ballistic trajectories we could show that the circuit indeed had internalized the laws of ballistics. Zebrafish were able to select a fine-tuned C-start for arbitrary initial motion conditions, even ones the fish had never encountered before. These findings thus offer the unique chance to study the buildup of a representation of a law of physics in a defined small network and to understand how laws of the external world are represented in the language of neuronal circuits.

# INTRODUCTION

Decision-making is a fundamental task in everyone's life. But the neuronal basis is still elusive. Complex decisions of the mammalian brain rely on complex networks with millions or even billions of neurons and many synaptic levels. Although, for instance, impressive advances in fMRI (functional magnetic resonance imaging) technology enable to study the human brain as it engages in complex decisions (e.g. Heekeren et al., 2004; Tom et al., 2007), these studies only allow linking the decision-making processes to certain rough areas or centres in the brain, instead to specific neurons. Another decision-making process, which, in fact, is one of the most influential paradigms in primate decision-making, is the saccadic eye-movement in trained monkeys (e.g. Glimcher, 2003; Gold and Shadlen, 2007; Wang, 2008; Churchland et al., 2008). Monkeys view a screen and make a saccade into the direction where the majority of dots presented move while specific areas of the brain are recorded extracellularly. Strikingly, units within the lateral intraparietal area (LIP) were discovered to play a decisive role in the decision. However, the mechanisms of decision-making that can be concluded of these studies still typically describe the interaction of centres, each representing far too many neurons to bring into reach an understanding of mechanisms at the cell level. A different approach to understand the mechanisms is to consider smaller and thus more accessible decision networks, for instance in leeches (Briggman et al., 2005, Birggman and Kristan, 2006). Their small number of only 10000 cells in the nervous system, each of them individually identifiable, makes leeches a good model system to understand the neuronal mechanisms when they select either to swim or to crawl, two mutually exclusive behaviours. Remarkably, individual cells were found to be the ‘decision-making’ cells. However, using voltage-sensitive dyes to monitor 80% of the neurons in a ganglion discovered that a small group of neurons predicted the leech’s choice prior to these ‘decision-makers’. The decision to either crawl or swim was not made by single cells, instead the decision correlated to the activity pattern of ensembles of neurons. Although the leech’s simple and accessible neuronal networks provide a profound insight in the mechanisms of decision-making, there still is the detriment of simple and even mutually exclusive behaviours.

A complex decision-making processes that even can be studied on a neuronal level is the C-start response shown by most escaping teleost fish (e.g. Eaton et al., 1977; Eaton et al., 2001). In order to gain the highest attainable acceleration, startled fish perform a unique and characteristic two-stage pattern. First, they bend into a C-shape in an opposing direction to the escape resource and then, rapidly push off as much water as possible with the full broadside of their body, all fins erected (Weihs, 1973; Webb, 1975). Surprisingly, a remarkably simple and small neuronal network of a few hundred reticulospinal neurons with few synaptic levels in the fish's hindbrain enables this quite complex and elaborate behaviour. In this network a set of six identified cells, one pair of Mauthner (M)-cells and its homologues, play a key role in driving the fast-starts. These pairs of cells can be determined from one individual to the next. Ablation experiments showed that, when they are removed, C-starts are still possible, but no longer at top speed and top performance (Kimmel et al., 1980; Liu and Fetcho, 1999). M-cells are of particular interest because they are the first to fire if a response is to be elicited to a startle stimulus, they are the largest neurons and the ones whose enormous axons conduct fastest. They determine whether the body will bend towards the left or right side with a single spike fired by one of them (e.g. Zottoli, 1977; Eaton et al., 1981). Moreover, all information essential to perform escape starts in the required direction, visual information together with input from the auditory, trigeminal, vestibular and lateral line system, is directly projected to the M-cells (Kimmel et al., 1981; Kimmel et al., 1990; Zottoli et al., 1987; Canfield, 2006; Mirjany and Faber, 2011; Mirjany et al., 2011). Because of its high accessibility (Furshpan and Furukawa, 1962; Faber and Korn, 1978) the neuronal networks of C-starts are one of the major model systems in neuroethology (e.g. Faber et al., 1989; Zottoli and Faber, 2000; Eaton et al., 2001; Korn and Faber, 2005).

Another form of C-start can be found in hunting archerfish of the genus *Toxotes*, which are renowned for their unique hunting technique (Fig. 1). They are able to shoot down aerial prey from substrates above the water with precisely aimed jets of water fired from their mouth (e.g. Smith, 1936; Lüling, 1963). Since the fish usually don't shoot from directly underneath the target, but more from lateral positions, dislodged prey falls on ballistic trajectories towards the water surface. However, downed prey could be readily lost to the more numerous other surface feeding fish seen in the wild. To reduce this risk, shooting comes packaged with a so-called predictive start (Schuster, 2012). Based on a brief sampling of the prey's initial movement (Schlegel and Schuster, 2008) – initial height, direction and speed – the shooter, but also bystanding other archerfish perform a C-start and take off in the direction of the prey's later point of impact long before the prey actually hits the surface. Predictive starts of archerfish share all hallmarks and the same

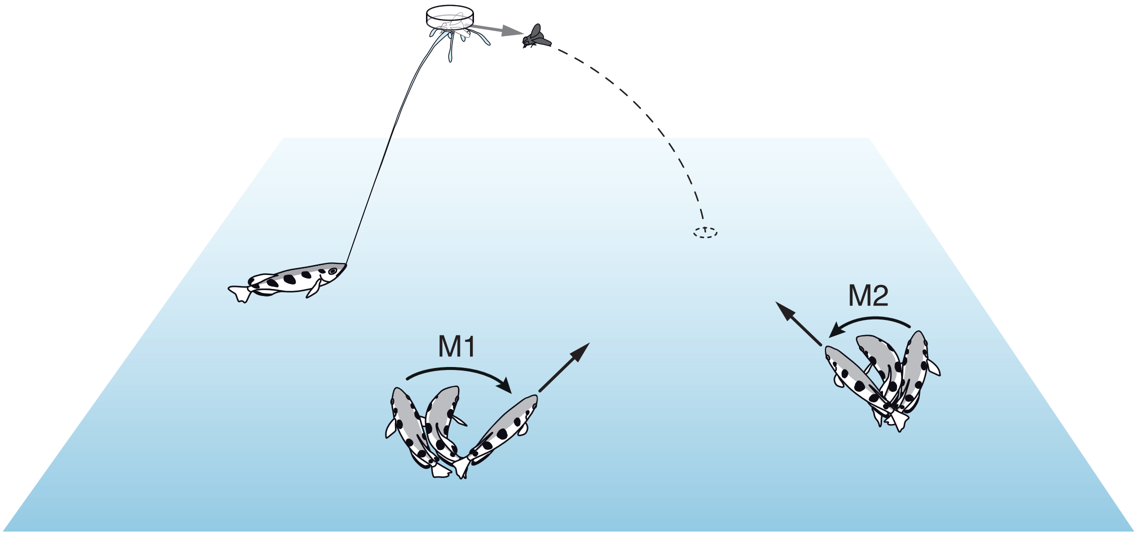

Fig. 1. Archerfish shoot down aerial prey with precisely aimed jets of water. Once dislodged, the prey falls on ballistic trajectories towards the water surface. Note, the prey can fall in every direction in the full angular range and with various speed levels. Together with height, direction and speed determine the later point of impact. Based on a short view of the prey's initial motion, archerfish select a C-start manoeuvre (M) that is matched to direction, distance and timing to the later point of impact to make the catch in time.

two-stage pattern as the M-cell associated escape C-starts mentioned above (Wöhl and Schuster, 2007) and are kinematically equivalent to archerfish escape C-starts (Fig. 2).

A short glimpse at the initial movement of the dislodged prey is sufficient for selecting the appropriate C-start manoeuvre – setting the direction (Rossel et al., 2002; Wöhl and Schuster, 2007; Schlegel and Schuster, 2008) and presumably also take-off speed attained immediately after the end of the C-start (Wöhl and Schuster, 2006; Reinel and Schuster, 2014). This ability appears to be needed in light of the severe competition: Missing or overshooting the point of impact could mean arriving later than a competitor. Even worse, starting fish risk becoming conspicuous to nearby aquatic or aerial predators without the prospect of a reward. Since performance of escape and predictive C-starts is equal, it is most likely that the same neuronal network, the M-cell associated reticulospinal network, drives both behaviours. An additional network for prey-driven starts could be an

Fig. 2. Comparison of (A) a predictive start with (B) an escape C-start. Both starts involved similar turns and were imaged at 500 frames $s^{-1}$; every second frame is shown. Colour of background behind fish silhouettes highlights initial bending phase (stage 1; dark blue) and propulsive phase (stage 2; light blue). Taken from Wöhl and Schuster, 2007.

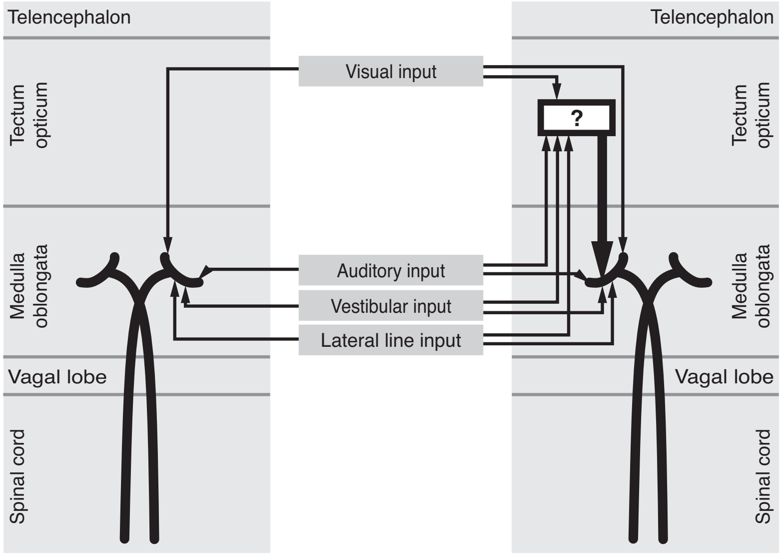

Fig. 3. Schematic to illustrate why the Mauthner-cells are responsible to make the decision and why escape C-start and predictive C-start are driven by the same neuronal circuitry. As is known, all information (visual, auditory, trigeminal [not shown], vestibular and lateral line system) essential to perform escape C-starts is directly projected to the M-cells that most likely decide in which direction to escape (left). For predictive C-starts an alternative network with an additional centre to process information could exist (right). In this network higher-level brain regions would be responsible for decision-making and the M-cells would just execute their orders. Yet, in this case, mechanosensory input, for instance, would – although being already available at the M-cell – have to be additionally projected into these higher-level regions. For reasons of economy, this alternative is quite unlikely. Even less likely is the alternative (not shown), that the decision does not altogether involve the M-cell. This would mean the existence of a second cell that is equally large as the M-cell (because of speed) and whose mere purpose is to simulate the M-cell.

alternative, but for reasons of expense, this seems quite unlikely (Fig. 3).

Decisions should not be compromised even when internal or external operating parameters change that would affect function. False decisions – in case of C-starts, selecting an inappropriate C-start manoeuvre (incorrect turn angle or missing the right timing) – could mean losing prey to competitors or even life or death. To achieve ‘functional stability’ the C-start network has to be plastic to compensate environmental perturbations. On this assumption the main question of this thesis originated: *How flexible is the Mauthner-cell mediated C-start network? More precisely, can the M-cell associated C-start circuitry be tuned to reach correct decisions under changed environmental conditions?*

To answer this question, predictive C-starts seem even better suited than escape C-starts for several reasons. i) Predictable accuracy. To accurately measure the effects of changes on accuracy and timing for any given constellation, one should exactly know what the intended output (i.e. turn angle) would be. But this is clearly not the case for escape C-starts. Escapes have to be unpredictable to predators and therefore to the experimenter.

Certainly, escapes have to be fast enough and directed away from the startle resource, however, this pins directionality down by not much more than a range of almost 180 deg. Therefore, it is impossible to measure small deviations in accuracy between desired and actual direction. This is totally different for predictive C-starts. At any given situation, the desired output is known. Predictive starts aim towards the prey's point of impact given by the combination of initial values of the prey's motion. Therefore, even small deviations from this known projection can be measured. ii) Tight experimental control. Whereas escape C-starts can be triggered by visual, auditory and mechanosensory stimuli or any combination of these, predictive C-starts are elicited solely on the basis of visual information. But this is rather beneficial than detrimental since it leaves tight experimental control over the predictive start. Archerfish don't need *a priori* information for their decision-making process. At first, one might assume that observing the shot could signal to pay attention to motion cues of the falling object or could perhaps even give information of possible trajectories. A previous study was able to demonstrate that this is clearly not the case and *a priori* information is not needed: Starts in which the fish were deprived from this putative information were as accurate and as fast as in the natural condition with the putative information being present (Schlegel and Schuster, 2008). This finding allows tight experimental control to measure the speed and complexity of the decision-making circuitry. iii) Intrinsic control. Archerfish's starts automatically offer an intrinsic control: because the fish are eager to shoot to dislodge their prey, shooting serves as a control that they elicit predictive C-starts only when they are actually motivated to hunt. Additionally, the dislodged prey serves as an immediate reward to stay motivated. The shot itself doesn't necessarily benefit the fish's decision-making process to elicit accurately set aims to the future point of catch. The prey can take off in every direction with various speed levels not known to bystanders but also the shooter before the target starts to move. But shooting provides an intrinsic control for unchanged motivation, the fish will dislodge prey only when they are willing to hunt, therefore releasing predictive starts only when they are motivated.

Most importantly, aside from these methodological advantages, tough ecological demands must have shaped mechanisms to ensure the functionality of the predictive starts. Archerfish have to cover considerable distances in their search for hunting grounds in their complex mangrove biotopes. Thereby, they face dramatic and rapid changes in light conditions or temperature over short periods of time. Yet, sampling the motion cues of the falling prey and tuning their C-starts accordingly is crucial: Starting too late and thus arriving too late, estimating the timing wrong and overshooting the prey's point of impact, but also launching an inaccurately aimed fast start and thereby having to correct en-route, will have drastic consequences, namely losing prey to competitors while risking to make themselves conspicuous to nearby or aerial predators. Consequently, it would probably be better for the fish to not start at all.

The overall question of the plasticity of the M-cell associated C-start circuitry was addressed in several experiments. Knowing the behavioural limits of the way C-start decisions can be adjusted is particularly interesting, because the underlying circuit would eventually allow us to study the cellular mechanisms that determine a decision.

(1) In poikilothermic animals inherent buffering mechanisms must be at work to compensate temperature changes to maintain their physiological functions even when changes in temperature drastically change cellular and molecular properties within the underlying circuits. To what extend is the predictive C-start buffered to maintain the high accuracy when temperature changes?

(2) What happens when accuracy of the predictive C-starts is bound to fail? Is it better not to start at all than to start with an inaccurately set aim? To address these questions we performed an experiment in which archerfish were compromised of the visual information given by their prey's trajectory. With enough lighting present, archerfish derive the parameters of their dislodged prey, select their turns accordingly and take off to the later point of impact long before the prey hits the water surface. But reducing light ultimately deteriorates the visual input to accurately select the appropriate start.

(3) Predictive C-type fast starts are not exclusive to archerfish. The Middle-American machaca, *Brycon guatemalensis*, a stream-living fish, shows a similar behaviour. A vast proportion of their diet consists of fruit, e.g. figs. Fig trees release their entire load within one or two days and adult machacas often assemble under these trees, face-on against the stream, and wait for fruits to fall. Just as with archerfish, as soon as a fruit starts to fall, machacas select a rapid C-start motor response to the later point of catch. Certainly, since figs fall down straight, the visual part of the problem is very much simpler for machacas than for archerfish that have to solve the complexity of the prey's ballistic trajectory. But for their stream-living nature, machacas experience heavy drift on their way to the impact point; their falling prey does not. Starting straight towards the later point of impact thus

would require continuous time and energy consuming course corrections. Alternatively, already at start, machacas could take the drift they will encounter on their way to the target into account in addition to the visual information given by the falling fruit. But is the C-start circuitry flexible enough to take this additional information into account?

(4) Consequently, the question evolved: Would it be possible to train adult zebrafish, *Danio rerio*, to tune their mechanosensory based C-start to a visual driven predictive start? More precisely, is it possible to tune the underlying circuitry in an animal that performs directed C-starts on the basis of mechanosensory information to incorporate visual information (previously decision-irrelevant) to select an adaptive C-start that rotates the responding fish so that it is likely to make the catch? At first, this hypothesis might sound absurd. But on the other hand it might be possible. Zebrafish have similar living conditions compared to archerfish or machacas: living in groups with high competition, surface-feeding with at least parts of their diet being aerial food, and high risk of predation. Even though all these species are only distinct related (Fig. 4), it could be demonstrated that the high ecological demand has driven the evolution to predictive fast starts at least in archerfish and machacas. Therefore and under the assumption that escape and predictive fast starts are based on the same neuronal network, it is plausible that zebrafish could learn to incorporate visual cues of falling prey in their C-start circuitry. This could even more support the plasticity of the M-cell associated C-start network to even incorporate novel cues that it was not able to handle before.

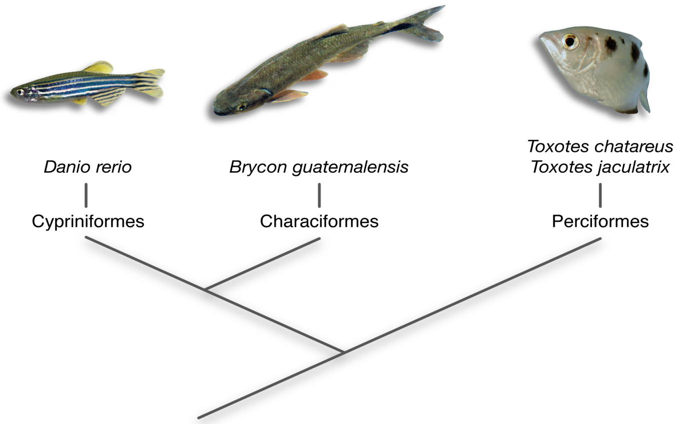

Fig. 4. Cladogram showing the relations between archerfish (*Toxotes* spp.), machacas (*Brycon guatemalensis*) and zebrafish (*Danio rerio*).

# SYNOPSIS

The central topic of my thesis was exploring the plasticity of the Mauthner (M)-cell associated C-start circuitry. This circuit drives the major escape response pattern of teleost fish, the so-called C-start, in which the fish first bends into the shape of a letter C and then rapidly pushes water backwards while straightening. A remarkable property of this circuitry is that it contains a comparably small number of neurons that not only are large but can be identified from one fish to the next. Among these neurons the Mauthner cell plays a key role. The two Mauthner cells not only are the largest cells in the circuit but they decide whether a start is launched or not. Due to their size these neurons have so far played a major role in neuroscience (Korn and Faber, 2005). The specific question addressed on the studies presented here was, *can the M-cell associated C-start circuitry be tuned to reach correct decisions under changed environmental conditions?* This is addressed here in behavioural experiments as a first step to then study the cellular mechanisms that mediate the plasticity.

In these studies a complex fast-start motor decision made by hunting archerfish (of the genus *Toxotes*) plays an important role: their so-called predictive C-start is also driven by the above-mentioned circuitry but is a precise motor decision among thousands of alternatives but made in less than a tenth of a second (Schuster, 2012). In their predictive starts the fish have to quickly estimate the initial movement of dislodged, ballistically falling prey and then select from a vast number of alternatives – at least 1000 alternatives if not more – the appropriate C-start manoeuvre that turns the fish right to the later landing point of their still falling prey (e.g. Schlegel and Schuster, 2008) and, moreover, that pushes them off with a matched speed to arrive at the right spot in just the right time (Wöhl and Schuster, 2006; Reinel and Schuster, in press). The high accuracy and the known target position of the starts allow a sensitive straight forward assay to detect even slightest deviations in the archerfish's decision upon fully controllable changed environmental conditions that could compromise performance, for instance temperature changes or when the visual input upon the decision is based gets less reliable by reduced light conditions. Together with the accessibility of the underlying circuits on a cellular level make the archerfish predictive starts a perfectly suited model systems to study how environmental perturbations affect the circuitry and how well C-starts can be functionally buffered. For better understanding, I arranged the contributions in a logical instead of chronological order.

The **first publication** *Precision of archerfish C-starts is fully temperature compensated* (*J. Exp. Biol.* 216, 3450-3460, 2013) asks if changes in temperature affect the performance of the archerfish's predictive C-starts. Temperature drastically changes cellular and molecular properties within the underlying circuits and of the entire physiology of poikilothermic animals. To maintain their vital physiological functions buffering mechanisms must be at work to compensate these changes. It has to be stressed that, in general, the decisions have to be correct even when environmental conditions change. This assumption certainly also applies to the archerfish's decision, releasing a predictive C-start that is incorrectly matched to position and timing of the future point of catch (incorrect turn angle or take-off speed) means loosing food, but seems to apply even more to the life or death decision of an escape. While it might sound obvious and trivial that decisions have to be correct even when conditions change, it can't be taken for granted or sometimes it simply can't be achieved. Two previous studies on the temperature dependency of escape starts seem to show a failure of the system to fully re-adjust after changes in temperature (Preuss and Faber, 2003; Szabo et al., 2008). While acute cooling increased behavioural responsiveness, it decreased directional selectivity, i.e. 'false' decisions increased, which could directly be linked to changes in the dendritic cable properties of the Mauthner-cell and to changes in the balance between excitatory and inhibitory inputs into the M-cell (Preuss and Faber, 2003). Particularly interesting, even after an acclimation period of 4 weeks, escape C-starts in goldfish were compensated for acute effects of cooling but not of warming up. Upon warming, an increase in escape probability and a decrease in directionality remained uncompensated, again explained at the level of the M-cell as a lack of compensation for increased excitatory synaptic inputs (Szabo et al., 2008).

Yet, it is easy to underrate the degree of functional stability in escape C-starts. Major experimental difficulties with escapes might mask their stability: (i) Escape starts have to be unpredictable to the observer and therefore involve a random element. Without independent evidence on the acute levels of this random element only approximate assays of functionality are possible. (ii) Because only rough changes in directionality can be detected in escapes, large changes in temperature are needed to see effects on directionality, but these changes may not always be in accordance to the normal operating range of the animal. (iii) It is difficult to control motivation. Assuming that the motivation to perform escapes should be constant might not be true when alternatives exist. ‘Freezing’ or simply suppressing the C-start could be better at lower temperatures than launching a C-start too late or with insufficient power to accelerate the fish fast enough away from a predator. But clearly, these kinds of alternatives diminish the escape start probability. This situation is completely different for archerfish C-starts. When archerfish are motivated, they hunt and dislodge prey and readily elicit their directed C-starts upon the initial movement of dislodged prey. As explained above, the ballistic trajectory of the falling prey defines the archerfish’s decision: C-starts are adapted to the initial motion of the target so that turn angle and initial speed are matched to the position and time of the future point of catch. Suppressing a start, which might be an optional change in strategy for escapes to hide from predators, or launching an erroneous start would mean loosing food to the numerous competitors. Moreover, archerfish encounter rapid temperature fluctuations in their mangrove biotopes that lead to the high ecological demand for temperature-compensated functionality. The high accuracy and the known target point of the archerfish predictive C-start should, therefore, reveal how temperature affects the underlying circuitry, ultimately disclosing inherent buffering capabilities of the neuronal circuit or the lack of them.

Surprisingly, a brief acclimation period of only one week between temperature changes sufficed to fully maintain the functional performance of the archerfish C-starts. Over the range of operating temperatures the fish were equally well motivated and full responsiveness was achieved. At all temperatures the fish accurately selected their turns to the future point of catch. Thereby the high accuracy was not obtained by restricting the angular range, instead, the fish operated over the full angular extent despite the changes in temperature. Most remarkably, the archerfish’s ability to judge distance to the future landing point and remaining time until impact was not compromised by changes in temperature. Their take-off speed immediately attained after the end of the C-start was equally well matched to the ratio of distance and remaining time at all temperatures. Despite the fact that precision of the predictive starts was fully temperature compensated – i.e. full responsiveness, high accuracy of aims, and take-off speed accurately set to distance and timing – C-start latency and kinematic aspects of the C-start were not. Latency increased by about 4 ms per 1°C cooling. C-start kinematics were only partly compensated for. While at lower temperatures the duration increased similar to latency, the duration of the two major phases of the C-start remained constant above 26°C. It might be tempting to speculate that latency increased to allow sampling of more information of the prey's ballistic trajectory in order to maintain high accuracy after cooling. This assumption holds true at a decreased visual contrast of prey motion relative to its background (Schlegel and Schuster, 2008) and at low light levels (see second publication below). But here, this was probably not the case for two reasons: first, latency changed with absolute temperature, but didn't change over the course of the experiments at one respective temperature, and second, the amount of change was similar for latency and turn kinematics, at least at lower temperatures in which turn kinematics were not compensated. Latency consists of many factors, e.g. the visual transduction amongst others, and all of these factors are temperature dependent (see Lenz et al., 2005).

Given that all properties were stable after a brief acclimation period of about one week, acclimation to a novel temperature must have been fast and sufficed to preserve the precision of the archerfish C-starts. These behavioural findings therefore suggest a remarkable capacity of the Mauthner-cell associated C-start networks to be 'functional stable', i.e. to provide precise functionality even when operating conditions change. In view of the neurophysiological accessibility of the underlying circuitry, the archerfish predictive start may therefore facilitate our future understanding of how this plasticity and functional stability is achieved on the cellular level.

Decisions must be correct even when conditions change. But what happens when the decision is bound to fail? Is it maybe better to decide not to engage in the situation than to decide incorrectly? Previous studies on the archerfish performance have typically been done in perfect lighting conditions. No matter how difficult the task was made it did not lead to incorrect decisions, i.e. erroneous aligned C-starts. Conflicted with two objects simultaneously with equal speed but in opposing directions, archerfish did not average the opposing motion signals (Schlegel and Schuster, 2008). Instead, the fish exploited selective attention as an integral part of their complex decision and immediately and highly selectively decided which of the two conflicting motion signals to take into account, completely ignoring the other. This decision was not made at random, rather the fish selected the object whose later landing point was closer to their start position. Surprisingly, this added decision neither altered precision nor did it prolong latency of the predictive start. On the other hand, reducing the contrast between target and background did not reduce accuracy, but lead to an increase in latency, most likely, to add additional time to sample more information to preserve accuracy (Schlegel and Schuster, 2008). In the **second publication** *Meta-control ensures maintained accuracy in high-speed decision-making* (to be submitted shortly), we wanted to go even further, to make the archerfish task of evaluating the prey's initial movement and selecting the appropriate C-start manoeuvre harder by reducing light while keeping the contrast constant.

What happens when it is impossible to achieve correct decisions? Ultimately, the reduction of light deteriorates the information needed to decide correctly. Decisions made based on less reliable input or when time is limited lead to a decrease in accuracy, i.e. the corrosion of accuracy in the sensory input is accompanied by a corrosion in output accuracy (e.g. Harris and Wolpert, 1998; Gold and Shadlen, 2007; Körding, 2007; Heitz and Schall, 2012; Brunton et al., 2013; Carandini and Churchland, 2013). Usually, most current studies of decision-making rely on two-alternatives forced choice experiments. The animal is not free to decide to engage or not to engage in the decision. Potential mechanisms might be neglected that could prevent erroneous decisions by controlling the expected quality of the decisions and for instance suppressing decisions that will be so inaccurate that they are unlikely to be useful or might even be harmful. Using the archerfish predictive start, we provide an example of a complex multi-alternative motor decision that is not restricted by two choices. Instead, as mentioned above, archerfish face hundreds of alternatives to choose from, to select the appropriate C-start manoeuvre that is matched in direction and speed to the later point of catch (Wöhl and Schuster, 2006; Krupczynski and Schuster, 2013; Reinel and Schuster, in press) and, moreover, with the possibility to decide not to engage in the decision at all. To fully control the visual input the archerfish predictive C-starts are based on, we used an arrangement that allowed for changes in light conditions while ensuring homogenous background lighting and keeping the contrast between target and background constant and independent of viewing angle within a large area of about 20 × 20 fish lengths.

Dimming the light below a critical level reduces the input reliability of visual information on which the archerfish motor decision must be based. Based on the findings of numerous other decision-making systems, we expected to see at some point erroneous decisions, i.e. accuracy should deteriorate. Surprisingly, this was clearly not the case at all. Despite dimming the light increased the latency and decreased the release probability, it didn't affect the accuracy of the predictive starts. Remarkably, over the range of 6 orders of magnitude of light levels down to $5\times10^{-3}$ cd m$^{-2}$, archerfish were able to initiate a C-start that turned them right towards the later landing point with equal precision over the full angular range. The fish thus did not make systematically smaller turns to antagonise the increasingly difficult task. Instead of eliciting erroneous aligned starts, the frequency of C-starts, i.e. the probability of deciding to engage in a decision, declined. Although the fish demonstrably could see and still dislodged prey below $5\times10^{-3}$ cd m$^{-2}$ and even far lower light levels, no C-start was released. While dimming increased latency and reduced the release probability, the basic kinematic properties of the C-starts were, however, unchanged. This is in clear contrast to results when the predictive starts were affected by temperature. Both, latency and turn kinematics increased in the same amount with cooling (Krupczynski and Schuster, 2013; see above). It is, therefore, reasonable to conclude that i) latency increased to maintain the high accuracy, ii) no additional sampling of the prey's trajectory was required during the turn and iii) the estimate of the accuracy of the decision was made before the starts were initiated

If a decision is made it is always accurate. For archerfish it seems preferable not to participate at all than to produce false decisions. This may be because prematurely producing an inaccurate predictive C-start – a false decision – would simply waste energy and, worse, increase the chances of attracting the many aquatic and aerial predators around in the field without being compensated with food. The ability of archerfish to assay accuracy before the decision is actually made is unusual and strongly contrasts with the key finding in many other decisions. Yet, the major difference between the archerfish's decisions and those studied otherwise is that the fish were free to decide not to engage in the decision. Our results suggest an efficient pre-release control mechanism of yet unknown basis that ensures accuracy, i.e. C-starts are released only if they are expected to be accurate. This mechanism would comply with all criteria of 'metacognition', i.e. the ability to decide to engage or not engage in a decision, based on its expected accuracy (Foote and Crystal, 2007; Foote and Crystal, 2012). We can only speculate on the neuronal substrate of this mechanism, but most likely it resides in the M-cell associated C-start circuitry or even at the single cell level, the pair of Mauthner-cells. The kinematic equivalence of the archerfish's predictive start and the M-cell associated escape start (Wöhl and Schuster, 2007) hint that the same network drives both behaviours. Moreover, the input structure onto the M-cells (Kimmel et al., 1981; Kimmel et al., 1990; Zottoli et al., 1987; Canfield, 2006; Mirjany and Faber, 2011; Mirjany et al., 2011) suggests that the pair of M-cells are the most likely candidates for playing the role of the veto-authority, to decide if accuracy can be achieved or not.

Another environmental factor that influences the decision-making process in which direction to start to reach a desired target point can be drift. This is described in the **third publication** *Fruit-catching fish tune their fast starts to compensate for drift* (*Curr. Biol.* 18, 1961-1965, 2008). Numerous animal navigators are known to cope with drift caused by winds and currents and compensatory mechanisms have mostly been studied in long-distance migrations (e.g. Papi and Luschi, 1996; Srygley, 2001; Thorup et al., 2003; Alerstam, 2006; Liechti 2006; Chapman et al., 2008). But dealing with drift is not exclusive for long-distance migrators. Bees, for instance, may encounter strong crosswinds on their search for food and still are capable of finding their way to the source and back (Riley et al., 1999; Riley at al., 2005). They achieve this on the basis of probing the optic flow of their initial movement and adjusting their movement so as to shift the retinal image of the environment accordingly to the desired direction.

The question arose if drift could be compensated for in the route planning of riverine fish. More specifically, would the M-cell initiated C-start circuitry fail to accomplish correctly aimed C-starts in prevailing drift? Alternatively, the neuronal network could incorporate a mechanism to compensate drift effects in the decision-making process right from the start even before the fish took off towards the target, rather than probing and adjusting the direction en route. If the fish could do this, this would mean that they solve the problem of drift right at their starts, a completely novel way of compensating drift. To address this question, studying the M-cell associated escape starts of most teleosts would be inappropriate. Escape starts have to be directed away from a startle source, but their specific target point is not known and, indeed, should not. Again the predictive starts of archerfish, introduced above, would be excellent models but analysing the impact of drift on the archerfish predictive starts is methodologically complicated and requires demanding flow channels. Yet, we discovered directed fast-starts in another species, the Middle-American fruit eating machaca, *Brycon guatemalensis*, which proved to be useful for investigating if drift could be compensated for in the M-cell associated C-starts.

Ripe fig trees along the shorelines of tropical rainforest rivers release all their fruit within a few days (Bannack et al., 2002). Machacas stay underneath these trees just beneath the water surface, head on against the stream. The schools never wait for a falling fruit to impact on the water surface, instead they respond to visual motion of a falling fruit by first initiating a rapid turn and then starting to move in the direction of the future landing point prior to the impact to arrive on time with their target. Certainly, to solve for the vertical trajectory of falling fruits is much simpler than predicting the ballistic trajectory archerfish have to be capable of, but machacas face prevailing drift on their way to the point of impact, which the falling fruits do not.

So far (until this publication) precisely tuned C-starts to a future point had only been known to occur in archerfish (Rossel et al., 2002, Wöhl and Schuster, 2007; Schlegel and Schuster, 2008). The directed fast-starts of machacas shared all hallmarks of a typical M-cell C-type fast-start, such as those commonly found in the rapid M-cell initiated escapes of most teleost fish (Domenici and Blake, 1997) or the precisely aimed predictive starts of archerfish (Wöhl and Schuster, 2007) and are therefore likely to be driven by the same M-cell associated reticulospinal neuronal network: The turn consisted of the typical two phases of C-starts, initiating the turn by bending into a typical C-shape and the propulsion phase by straightening the body, but additionally, their turn duration, average turning speed, and linear acceleration resembled typical M-cell C-starts. Interestingly, any form of aerial motion did not trigger the early starts unspecifically; to elicit a fast start required a downward component, whereas an upward acceleration completely failed. Intriguingly, stopping a target before it touched the water surface neither altered responsiveness nor turn kinematics and accuracy of the post-start alignment towards the future point of impact. This finding together with the findings that the fish kept their initial speed and direction constant – surprisingly even in starts which were completely erroneous – indicated that the fish set their motor program already before the beginning of the C-start, i.e. the turns were executed in an 'open-loop' mode, another characteristic also shared by the archerfish predictive C-starts.

However, the question remained open where the fish aimed at. Did the fish incorporate the prevailing severe relative drift they encountered *en route* into their decision-making process before they even started? While the fruit falls freely in air, the fish have to cope with drift on their way to the fruit through rapidly flowing water. Aiming directly at the later point of impact would have the drawbacks of continuously steering against the flow, finally leading to curved trajectories and prolonged travel routes. On the other hand, accounting for the flow by selecting an optimal upstream heading relative to the beeline would lead straight to the target, thus, speeding up travel time and minimising costs.

Remarkably, the fish indeed chose a heading that was already optimally adapted to the prevailing drift, so as to lead them on a straight trajectory to their food. How did they achieve this? Did they simply apply the same upstream correction regardless of where they started? This was clearly not the case and also not feasible. Just as expected, fish starting from more lateral positions in regard to the target position used significantly larger drift corrections than the fish when the impact point was located directly upstream in front of them. Moreover, the fish were able to choose to apply or not to apply a drift correction. Very distant fish that started after their food had landed, aimed straight at the target's actual position. In this case, the fruit experienced approximately the same drift as the fish, so a drift correction was needless.

Machacas set their optimal initial heading right from the start to compensate for the prevailing drift. Hence, they had made their decision based on information sampled before they initiated their turns. Sensory feedback could not play a decisive role, which is in contrast to the mechanism used by bees, for instance (Riley et a., 1999, Riley et al., 2005), indicating, thus, a novel mechanism to compensate for drift. Moreover, the incorporation of this information into their decision-making process clearly demonstrates the capability of the M-cell associated C-start circuitry to adjust to environmental perturbations.

Archerfish have been long known to elicit visual driven C-starts to reach their goal before the target hits the water surface (Rossel et al., 2002). Yet, more recently another fish species, the fruit-eating machaca, was discovered to also drive their directed fast-starts based on visual information of their prey (Krupczynski and Schuster, 2008). The same neuronal network that is responsible for escape C-starts, the M-cell circuitry, is likely to underlie the starts of both species (Wöhl and Schuster, 2007). Both fishes share similar tough ecological demands and the vast majority of their diet consists of aerial prey. Their ability to produce a directed C-start based on visual information and to be on the way to their target before it hits the water surface and thereby alarms non-visual competitors is a clear benefit. The fact that these starts evolved in two only distinct related species led to the question, if it was possible to tune the underlying circuitry in an adult animal that initially performs directed C-starts solely on the basis of mechanosensory information to incorporate visual information in the decision-making process. This is part of the **fourth publication** *Full adaptive plasticity in a vital circuit of the adult vertebrate brain* (*Nature*, submitted).

Zebrafish, *Danio rerio*, can make use of mechanosensory information of the splashing impact to direct rapid C-starts towards a food object. Visual information of the food's trajectory towards the water surface is thereby decision-irrelevant. Although it might seem paradoxical at first sight that the underlying neuronal network can be tuned to incorporate visual cues to the decision, several convincing reasons exist. Zebrafish share similar ecological demands with archerfish and machacas. All three species live in schools with high intra- but also interspecific competition and with high risk of predation. Furthermore, their feeding habit is quite comparable. Whereas archerfish and machacas are primarily dependent on aerial food, zebrafish are likewise surface-feeding and at least part of their diet consists of aerial insects (McClure et al., 2006; Spence et al., 2007). Under the assumption that tough ecological demands and the clear advantage over competitors led to the evolution of visually guided C-starts and the further assumption that the same neuronal circuitry is responsible for the mechanosensory as well as visually based starts (Wöhl and Schuster, 2007), it is plausible that the network of zebrafish can be tuned to incorporate visual cues of falling prey, just as the underlying neuronal circuitry was able to be tuned to compensate for drift (Krupczynski and Schuster, 2008; see above).

Extensively exposing zebrafish to an environment in which the only available food source was aerial and fell on ballistic trajectories, indeed, tuned the previous mechanosensory based rapid motor decision to incorporate visual information to select a fine-tuned fast-start. The C-start turned the fish perfectly aligned towards the point of catch before the food impacted on the water surface that no further correction en route was needed. Remarkably, their starts did not account for further details such as spatial characteristics of the ballistic trajectory, instead, C-starts were driven by the minimum amount of information required by physics – height, direction and speed of the initial motion. Challenged by arbitrary initial motion conditions, even novel settings the fish had not previously encountered, revealed that zebrafish had internalized the falling laws that allowed immediate generalization to novel situations rather than operating on memory templates, i.e. interpolating among existing stored C-start patterns which start to produce for a given constellation of target motion and initial orientation of the fish to make the best possible choice in a given situation. The visually tuned C-starts showed a further remarkable characteristic. When the fish were challenged with two food objects simultaneously taking off in opposing directions the fish did not average the initial motion parameters. Instead, they rapidly decided to select one of the two constellations and elicited a C-start towards the chosen point of catch. The fish thus completely ignored the set of initial motion values that corresponded to the other object. Surprisingly, this added decision neither affected latency nor accuracy of the C-starts. Furthermore, their choice was not made at random: While distance did not play a role in the selection, the fish chose on the basis of turn size, selecting that of the two objects that required the lesser turn.

The substantial similarity in pre- and post-visually tuned C-start kinematics together with the level of performance firmly suggests that both decisions were mediated by the same defined cellular accessible motor network, the M-cell associated C-start circuitry. Furthermore, the visual tuning did not ‘overwrite’ the mechanosensory tuning and visually tuned fish were able to elicit appropriate starts based on both cues. While the C-starts driving reticulospinal circuitry initially could not handle to incorporate visual information into the decision-making process, it could be tuned to a remarkable level of performance even in the adult brain. This suggests that part of the adjustment is imprinted in the M-cell associated circuitry.

These findings strikingly demonstrate the plasticity of the Mauthner-neuron associated C-start circuitry, not only to buffer environmental perturbations, but also to incorporate novel information and to even represent a law of the external world which had been previously decision-irrelevant. The accessibility of the defined small circuitry together with the techniques available in zebrafish bring within reach to disclose the cellular mechanisms by which neuronal circuits represent laws of the external world and which factors support the incorporation of novel laws in the adult brain. Zebrafish may thus facilitate our understanding how learning takes place on a cellular level, even in the adult brain.

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# AUTHOR'S CONTRIBUTION

(1) **Krupczynski, P. and Schuster, S.** (2013). Precision of archerfish C-starts is fully temperature compensated. *J. Exp. Biol.* **216**, 3450-3460. http://dx.doi.org/10.1242/jeb.088856

Stefan Schuster and I conceived the project. I designed and built the experimental setup and conducted the experiments. Stefan Schuster and I analysed the data and wrote the manuscript. Personal contribution: 90 %

(2) **Krupczynski, P. and Schuster, S.** (to be submitted shortly). Meta-control ensures maintained accuracy in high-speed decision-making.

Stefan Schuster and I conceived the project. I designed and built the experimental setup and conducted the experiments. Stefan Schuster and I analysed the data and wrote the manuscript. Personal contribution: 90%

(3) **Krupczynski, P. and Schuster, S.** (2008). Fruit-catching fish tune their fast starts to compensate for drift. *Curr. Biol.* **18**, 1961-1965. http://dx.doi.org/10.1016/j.cub.2008.10.066

Stefan Schuster and I conceived the project. I designed and built the experimental setups and conducted the experiments in the tropical rainforest at the Biological Station La Selva in Costa Rica. Stefan Schuster and I analysed the data and wrote the manuscript. Personal contribution: 90%

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Stefan Schuster conceived the study. Romy Böhme, Sönke Ahlf, Marc Volkmar and I performed the experiments. All authors analysed data. Romy Böhme and I wrote algorithms. Stefan Schuster and I wrote the manuscript. Personal contribution: 30%

# PUBLICATIONS

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## RESEARCH ARTICLE

# Precision of archerfish C-starts is fully temperature compensated

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## SUMMARY

Hunting archerfish precisely adapt their predictive C-starts to the initial movement of dislodged prey so that turn angle and initial speed are matched to the place and time of the later point of catch. The high accuracy and the known target point of the starts allow a sensitive straightforward assay of how temperature affects the underlying circuits. Furthermore, archerfish face rapid temperature fluctuations in their mangrove biotopes that could compromise performance. Here, we show that after a brief acclimation period the function of the C-starts was fully maintained over a range of operating temperatures: (i) full responsiveness was maintained at all temperatures, (ii) at all temperatures the fish selected accurate turns and were able to do so over the full angular range, (iii) at all temperatures speed attained immediately after the end of the C-start was matched – with equal accuracy – to 'virtual speed', i.e. the ratio of remaining distance to the future landing point and remaining time. While precision was fully temperature compensated, C-start latency was not and increased by about 4 ms per 1°C cooling. Also, kinematic aspects of the C-start were only partly temperature compensated. Above 26°C, the duration of the two major phases of the C-start were temperature compensated. At lower temperatures, however, durations increased similar to latency. Given the accessibility of the underlying networks, the archerfish predictive start should be an excellent model to assay the degree of plasticity and functional stability of C-start motor patterns.

Key words: M-cell network, acclimation, motor circuit, functional stability, circuit homeostasis.

## INTRODUCTION

A fundamental integrative property of poikilothermic animals is that they manage to breathe, escape and maintain other vital functions even when changes in temperature drastically change cellular and molecular properties within the underlying circuits. An excellent example of a vital motor response that should not be compromised by temperature effects is the so-called C-start shown by most escaping teleost fish (e.g. Eaton et al., 1977; Eaton et al., 2001). This characteristic pattern allows fish to achieve the highest attainable acceleration by first bending their body into the shape of a letter C and then, with all fins erected, pushing off as much water as possible in as little time as possible (Weihs, 1973; Webb, 1975). Evidently, the fish must be able to keep the level of acceleration, short latency and directionality adaptive even when a change in temperature affects the elements of the underlying circuitry. In connection with the robust and vital behaviour they mediate, the accessibility of the underlying circuits (Furshpan and Furukawa, 1962; Faber and Korn, 1978) has made the C-start networks of fish one of the major models and sources of insight in neuroethology (e.g. Faber et al., 1989; Zottoli and Faber, 2000; Eaton et al., 2001; Korn and Faber, 2005). C-starts are driven by a surprisingly small set of a few hundred reticulospinal neurons in the hindbrain of the fish. Among these a set of six identified cells, which can be recognized from one fish to the next, play a major role in enabling the very fastest starts. When these are killed, C-starts are still possible, but no longer at the very top performance level (Liu and Fetcho, 1999; Kimmel et al., 1980). Among these, the pair of Mauthner (M-) cells is particularly interesting: in the intact system the M-cells are the largest, fastest conducting and first neurons to respond to a startle stimulus. A single spike in one of the two cells releases the C-start pattern and determines whether the body will bend towards the left or right side (e.g. Zottoli, 1977; Eaton et al., 1981).

Taking full advantage of this experimentally amenable system, two previous studies (Preuss and Faber, 2003; Szabo et al., 2008) succeeded in analysing both acute and acclimation effects of temperature on escape probability and directionality, and in relating these to cellular and synaptic changes at the level of the M-cell and associated circuitry. They thereby not only provided the first evidence for compensation effects in the vertebrate central nervous system but also linked them to properties of an identified neuron. Acute cooling increased behavioural responsiveness and decreased directional selectivity and this could directly be explained by changes in the dendritic cable properties of the M-cell and by changes in the balance between excitatory and inhibitory inputs into the cell (Preuss and Faber, 2003). A particularly interesting finding was that acclimation allowed the fish to compensate for the acute effects of cooling, but not of warming up: a drastic increase in escape probability and decrease in directionality upon warming was not compensated for. Again, the finding could be explained at the level of the M-cell as a lack of compensation for the increased excitatory synaptic inputs (Szabo et al., 2008). Environmental temperature affects the M-cell system in many ways and can even elicit M-cell spiking (Sillar and Robertson, 2009).

The accessibility of the underlying networks makes C-starts highly attractive for studying how well function is balanced against environmental perturbations. However, C-starts seem to lack one major ingredient of a good model: to accurately measure the effects of temperature changes on accuracy, one should know exactly what the intended output (i.e. turn angle, speed after C-start) would be in the given situation. If this is not known it is not possible to detect small deviations from the ‘desired’ pattern. Evidently, this is at odds with the very nature of an escape: all that should be known *a priori* – both to a predator and to the experimenter – is that the C-start should probably be directed away from the source. This means that the intended direction of an escape can only be known with an ~180 deg accuracy, making it impossible to detect small deviations between desired and actual direction in a given situation. Furthermore, although the escape should be fast, no study has yet identified exactly what speed the escape should initially have in a given situation. Most importantly, for an escape, variability in angle and speed in response to the same situation would seem to be an advantage rather than being detrimental. This degree of inherent and required variability would seem to prevent the experimenter from knowing precisely what the animal should do in a given test situation and to then precisely quantify the errors brought about by changes in temperature.

The situation is completely different with a newly discovered type of C-start shown by hunting archerfish (Fig. 1). Briefly, these fish dislodge aerial prey items with a shot of water fired from their mouth (e.g. Smith, 1936; Lüling, 1963). To increase the chance of actually catching the dislodged prey despite the presence of competitors, shooting comes packaged with a C-start motor pattern, the ‘predictive start’: after a brief view of the initial motion of the falling prey, the fish can initiate a C-start that turns them right towards the later landing point and pushes them off with a matched speed so that the fish would arrive at the right spot in just the right time (e.g. Schuster, 2012). With its aim well known for a

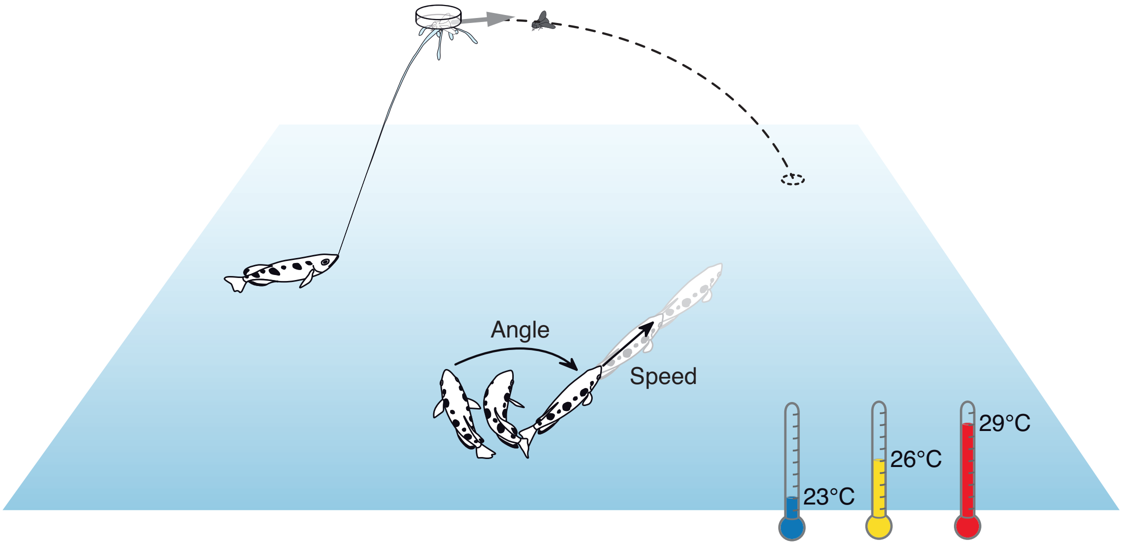

Fig. 1. The archerfish predictive start as a sensitive assay of temperature compensation in C-starts. Shortly after aerial prey is dislodged by a shot, archerfish can initiate a C-start, their so-called predictive start. The kinematics of this start are selected based on information sampled during the initial motion of the falling prey, from which the fish derive height, direction and speed and predict where prey is going to land. By adjusting their C-start accordingly, the fish rotate by just the correct angle and start with an appropriate speed that is matched to distance and timing. Because the required speed and aim at the end of the C-start is known for any combination, even small deviations of actual performance can be sensitively detected. In the tests reported here, a change in average alignment or an increase in scatter of the alignment below 1 deg could have been detected. Moreover, the fine-tuned archerfish C-starts provide an intrinsic control for unchanged motivation: the fish will dislodge prey only when they are in the mood to hunt and to catch prey. Thus, they provide the cues that release predictive starts only when they are motivated. The inset introduces colours used in all subsequent figures for the three main experimental temperatures 23, 26 and 29°C.

given combination of initial values of prey motion, the archerfish ‘predictive’ C-start would appear to be perfectly suited to study how well C-starts can be buffered against even slight changes in internal operating parameters that would affect function. The high accuracy attainable by studying archerfish C-starts could thus disclose an inherent buffering capability of C-start circuitry that very likely is also present in escapes but masked by our ignorance of the ‘randomizing’ additions the fish presumably adds to make its course unpredictable. Most importantly, the methodological advantages of using archerfish predictive C-starts come together with tough ecological demands for temperature-compensated functionality at all operating temperatures: their complex mangrove biotopes force the fish to cover considerable distances in their search for hunting grounds. The fish consequently often face dramatic and rapid changes in light conditions, salinity and temperature over the course of a few hours (S.S., unpublished observations). Shooting without a predictive start or with one that is not well aimed would usually mean losing the food to the more numerous competitors. Moreover, because the rapid starts are performed right below the water surface, they are conspicuous to the many aquatic and aerial predators around. So, it would probably be better for the fish to not start at all than launch an inaccurately aimed predictive C-start that misses the later landing point.

Using the methodological advantages of the archerfish predictive starts and the high constraints on precision, we explored the degree to which C-start manoeuvres can be functionally buffered against changes in temperature. Specifically, we analysed whether the fish can keep up the constant high release probability, fine-tuned selection of speed and angle, short latency and speed of the C-start motor pattern throughout the temperature range at which they hunt in the wild.

## MATERIALS AND METHODS

### Animals

Experiments were performed on a group of five archerfish, *Toxotes chatareus* Hamilton 1822. The body lengths of the fish (from snout to caudal peduncle) ranged from 7.6 to 10.9 cm (9.1±0.7 cm, mean ± s.d.). Fish were kept and all experiments were carried out in a large tank (1.0×1.0×0.6 m) filled to a height of 35 cm with brackish water (conductivity 3.5–3.7 mS cm$^{-1}$). A light regime of 12 h:12 h was maintained and experiments were started no earlier than 5 h after light onset. Because all group members responded to dislodged prey, their responses were pooled.

### Experimental setup and acclimation

The basic design is illustrated in Fig. 1. One dead fly (*Calliphora* sp.) at a time was wetted and stuck on the lower side of a transparent acrylic circular platform (50 mm in diameter), mounted 30 cm above the water surface in the centre of the tank. Dislodged flies fell ballistically (see Rossel et al., 2002). Experiments started at a temperature of 26°C. After 15 days of testing, the temperature was changed to 23°C, followed by 29°C and then – as a final control for stability of the general performance – an additional phase of 26°C. Each phase started with 7–10 days of acclimation in which the fish were kept in training. After this time, experiments were run for 3–4 weeks. To ensure comparable motivation, the number of trials run per day differed at the different temperatures: 30 trials per day at 26°C, 20 trials per day at 23°C and 35 trials per day at 29°C. Accordingly, the fish were tested for 15 days at 26°C, 27 days at 23°C, 21 days at 29°C and then another 15 days at

26°C. By performing the experiments in a temperature-stabilized room, whose temperature was accurately set 2°C below the tank's intended temperature, a 300 W heater sufficed to set temperatures with an accuracy of ≤0.1°C.

### Recording

We used digital high-speed video recording at 500 frames $s^{-1}$ (HotShot 1280M, NAC Deutschland, Stuttgart, Germany; lens Sigma 20 mm f/1.8 EX DG). The camera viewed the water surface from a height of 1.5 m at a right angle. Contrast was optimized by diffusely illuminating the tank from below by shining four halogen spotlights (200 W each) onto a diffusor plate (mounted below the tank to cover its full area). Additionally, two halogen spotlights (400 W each) were directed upwards onto a sheet that covered 2.0×2.0 m at a height of 1.8 m above the water surface. The resulting Michelson contrast between the falling fly (reflected light 31.3 cd $m^{-2}$) and its background (reflected light 422.4 cd $m^{-2}$) was 0.86 in all experiments. Because target and fish were at different distances, we needed to correct for the resulting metric distortions. To achieve this, we projected the falling prey onto the water surface, taking into account the known distortion in perspective that applied for the fly's current height level. From the way flies fell, the height level that we needed for this calculation could simply be derived from the time of falling, a procedure also used previously (e.g. Rossel et al., 2002; Schlegel and Schuster, 2008).

### Data analysis

The recordings were processed using ImageJ (developed at the National Institutes of Health) and custom-written software. We evaluated latency, aspects of the C-start kinematics, and the accuracy of the fish's rapid predictive turn and its take-off speed. Responsiveness was 100% at all temperatures, i.e. in all situations in which one group member fired at the prey and dislodged it, at least one group member showed a C-start that was finished before prey impact. To ensure that the C-starts were selected solely on the basis of information on prey motion but not on the basis of the responses of the other fish, we analysed only the C-start of the fish that responded first, as described previously (e.g. Rossel et al., 2002; Wöhl and Schuster, 2007; Schlegel and Schuster, 2008). To ensure that accuracy was not due to additional mechanosensory input from the splashing impact of prey, all analyses (including the take-off part that immediately followed the C-start) exclusively relate to C-starts after which the fish took off before the prey's impact. Because obstacles strongly influence the fish's initial turn (S. Wöhl and S.S., unpublished), we excluded cases in which another fish blocked the direct path to the later impact point. In order to easily measure latency and to critically assay turning accuracy, we required the starts to turn the fish by at least 10 deg. In a number of trials the dislodged fly simply fell vertically – these trials were not included in the present analysis because 'predicting' the later landing point would have been trivial in such cases. In addition, cases were excluded in which the fish could simply follow the prey's motion; a minimum angle of 10 deg was required between the fish's path to the point of impact and the fly's horizontal trajectory. Latency was derived from the number of frames between the onset of prey movement and the initiation of the C-start. Duration of the C-start and its two stages were similarly obtained. Stage 1 was defined as the time the fish needed from initiating the turn until its body was maximally bent, and stage 2 as the time from maximum bend until the end of the turn leading to the subsequent take-off phase. Accuracy of the turn was assessed as follows: the error e of the aim of the C-start was taken at the end of stage 2 as the minimum distance a line in the initial direction had from the fly's later landing point. As in earlier papers (e.g. Rossel et al., 2002), this distance was considered negative if the line intersected the projected path of the fly before the impact point; otherwise, it was taken as positive. The following analysis gave the accuracy of the speed adjustment: immediately at the end of stage 2, speed values were taken from the change in position of the fish's centre of mass (see Wöhl and Schuster, 2006) in four consecutive 10 ms intervals. Unless stated otherwise, the mean of these four speed values is reported.

### Statistical analysis

Unless stated otherwise all tests were run using SigmaPlot (version 11.0, Systat Software Inc. 2008) and performed two-tailed with an alpha level of $P$=0.05. In *post hoc* tests, the level of significance was examined using sequential step-down Bonferroni corrections. Normality of data was assayed using Shapiro–Wilk tests and additionally confirmed with Q–Q plots coupled with histograms. For parametric data, equal variance was checked using Levene tests. As non-parametric tests (for latency and C-turn properties), we used Mann–Whitney $U$-tests and Kruskal–Wallis one-way ANOVA on ranks, *post hoc* tested with Dunn's method. Parametric tests (for error in aim and take-off speed) consisted of one-sample and two-sample $t$-tests and one-way ANOVA, *post hoc* tested with the Holm–

Sidak method, and repeated measures ANOVA. Correlations were tested either using Pearson correlation (parametric) or Spearman rank correlation (non-parametric).

Tests on how temperature affected the relationship between take-off speed and 'virtual speed' were run in R (version 2.10.1, R Development Core Team, 2009) using multivariate linear models. To compare whether 'virtual speed' (i.e. remaining distance per remaining time) or distance by itself better describes the variability in take-off speed, we used two different multivariate linear models with either 'virtual speed' or distance as predictor. Model selection was based on the method described previously (Rödel et al., 2004; Burnham and Anderson, 1998; Wagenmakers and Farrell, 2004) using the second-order Akaike's information criterion (AICc). In summary, the most suitable model is the one with the smaller $\Delta_i$ (=AICc–minAICc) and the larger Akaike weights $w_i$. Additionally, R was used to test equal variance of non-parametric data using rank-based modified Brown–Forsythe tests.

### Stability

Table 1 reports all aspects of the C-starts both for the initial phase at 26°C and in the final control phase at the same temperature. None of the aspects showed any significant changes (see Results for an explanation of the meaning of the respective quantities). Hence, the general properties of the C-starts had not changed over the course of the present experiments, as would have been possible, in principle, by changes in social structure within the group or growth of the fish during the study period. Values subsequently reported for 26°C relate to pooled data from the first and final phase at 26°C.

Table 1. Comparison of initial and final measurements at 26°C demonstrates stability of overall conditions

| Variable | Initial | | Final | | Statistics |
|---|---|---|---|---|---|
| Latency (ms) | 73.9±14.6 | (91) | 72.4±15.6 | (104) | *U*-test *P*=0.28 |
| Size of turn (deg) | 43.6±24.5 | (91) | 41.7±24.6 | (104) | *U*-test *P*=0.50 |
| Total turn duration (ms) | 49.8±17.8 | (91) | 49.6±16.2 | (104) | *U*-test *P*=0.95 |
| Stage 1 duration (ms) | 19.7±5.2 | (91) | 20.5±6.4 | (104) | *U*-test *P*=0.36 |
| Stage 2 duration (ms) | 30.1±14.8 | (91) | 29.1±11.3 | (104) | *U*-test *P*=0.96 |
| Error e (mm) | –2.70±1.59 | (91) | 0.05±2.09 | (104) | *t*-test *P*=0.31 |
| Take-off speed (m $s^{-1}$) | 1.095±0.287 | (89) | 1.130±0.296 | (101) | *t*-test *P*=0.41 |

Initial and final values are shown as means ± s.d. with *N* in parentheses

## RESULTS

### A 100% release probability is maintained over the full range of temperatures

Because the fish had to actively dislodge prey, the experiments provided an automatic intrinsic control that the fish were motivated to hunt and to capture prey. Fish fired and successfully dislodged prey at all temperatures examined. Most surprisingly, however, whenever a target was dislodged, its initial motion always sufficed to release a predictive start of at least one of the fish that brought the fish on course before the prey had landed. A remarkable 100% release rate of predictive starts held at all temperatures: of 119 cases at 23°C, 119 released a predictive C-start. Similarly, each of 115 presentations at 29°C released a C-start and so did all 195 presentations at 26°C. The success rate of the first responder was also constant (75.2%, 79.5% and 72.6% at 23, 26 and 29°C, respectively). Furthermore, both the number of fish that managed to start quickly enough as well as the probability that the shooter was among them were remarkably unaffected by temperature: an average of three (2.98, 3.05 and 2.86 fish at 23, 26 and 29°C, respectively) of the five fish managed to be on their way at least 40 ms before prey impact. The probability that the fish that had actually fired the shot was among them was 87.4%, 87.2% and 87.8% at 23, 26 and 29°C, respectively. This lack of failure strongly contrasts with findings on the temperature effect on release probability in escape C-starts (e.g. Szabo et al., 2008).

### Acclimation is fast

An acclimation phase of about 1 week at a novel temperature was thus sufficient to ensure constant responsiveness of the fish. Surprisingly, the same held true for all parameters we examined in this study. Table 2 reports this for three major parameters that will be described in detail below: two (latency and duration of the C-start) that are not fully temperature compensated and one (alignment after C-start) that is perfectly temperature compensated. All three parameters showed no tendency to change during the testing period

Table 2. Latency, accuracy and duration of the C-start remained constant after the brief acclimation period

| Temperature | Latency | Error e | Turn duration |
|---|---|---|---|
| 23°C (*N*=119) | $r_s$=0.038, *P*=0.69 | *r*=0.148, *P*=0.11 | $r_s$=0.115, *P*=0.21 |
| 26°C (*N*=195) | $r_s$=–0.068, *P*=0.34 | *r*=0.053, *P*=0.49 | $r_s$=–0.025, *P*=0.73 |
| 29°C (*N*=115) | $r_s$=0.134, *P*=0.15 | *r*=–0.080, *P*=0.40 | $r_s$=–0.056, *P*=0.56 |

Data are the correlation of latency, accuracy and duration with time.

that followed the 1 week acclimation. The brief acclimation time at the novel temperature generally left the fish fully acclimated in all aspects of their predictive C-starts that we analysed in this study. The only aspect whose stability we were unable to test was the precision in judging distance and timing, and setting take-off speed accordingly. To assay this capability requires a large dataset of starts assembled over an extended time, thus restricting the temporal resolution at which a change could have been detected. Nevertheless, it would have had to occur rapidly, and be completed in the ~4 week testing phase. However, we were able to follow the course of changes in each of the other properties that we will describe throughout this paper and none of them showed a significant trend over time.

## Latency is not temperature compensated

While responsiveness remained constant at all temperatures, latency, i.e. the time between the onset of prey movement and onset of the C-start, increased with decreasing temperature (Fig. 2; Kruskal- Wallis: $H$=189.75, d.f.=2, $P$<0.001, Dunn's test: all pairwise $P$<0.05). Moreover, there was no indication that further acclimation would reduce this shift in latency (Table 2). In the range from 29 to 23°C, median latency increased about 4 ms per 1°C cooling. Besides its effect on median latency, cooling also affected the shape of

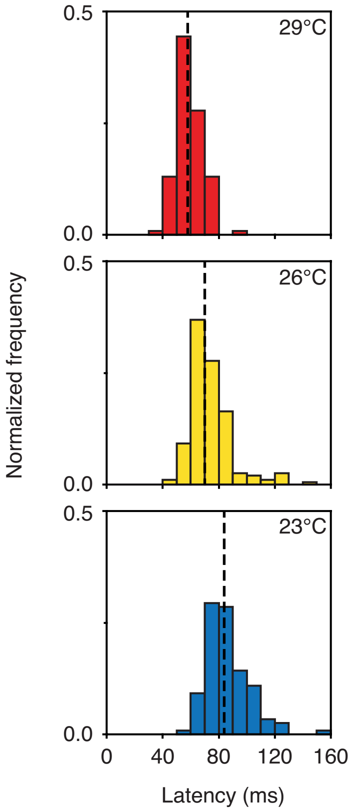

Fig. 2. Latency is not temperature compensated. Fish took less than 1 week to acclimate to the experimental temperature, after which time latency values were constant. Median latency (dashed lines) increased by about 12 ms with a 3°C cooling (Kruskal–Wallis: $H$=189.75, d.f.=2, $P$<0.001, Dunn's test: all pairwise $P$<0.05). Cooling also broadened the latency distributions, an effect that cannot be attributed to a changed internal motivation of the fish. Histograms are based on $N$=119, 195 and 115 responses at 23, 26 and 29°C, respectively, and normalized so that each total frequency equals 1. Binning starts at zero with bin widths of 10 ms.

the latency distributions: whereas the distribution was sharply focused at 29°C, it became slightly broader at lower temperatures (rank-based Brown–Forsythe: *P*=0.004). In addition, the distributions appeared to be more skewed at lower temperatures, an impression that is compatible with calculations of higher moments of the distribution: skewness increased from 0.5 at 29°C to 1.4 at 23°C and kurtosis increased from 0.6 to 3.7.

### Kinematics of the C-start manoeuvre change with temperature

After the brief acclimation period the temporal aspects of the C-start manoeuvre were also stable, with no indication of any further changes during testing. The kinematics were only partially temperature compensated (Fig. 3). The total duration of the C-start manoeuvre was significantly longer at 23°C than at both 26 and 29°C, but not significantly different between the two higher temperatures (Fig. 3A; Kruskal–Wallis: *H*=17.60, d.f.=2, *P*<0.001, Dunn's test: *P*>0.05 for 26 *versus* 29°C, *P*<0.05 for 23°C *versus* 26 and 29°C). The same

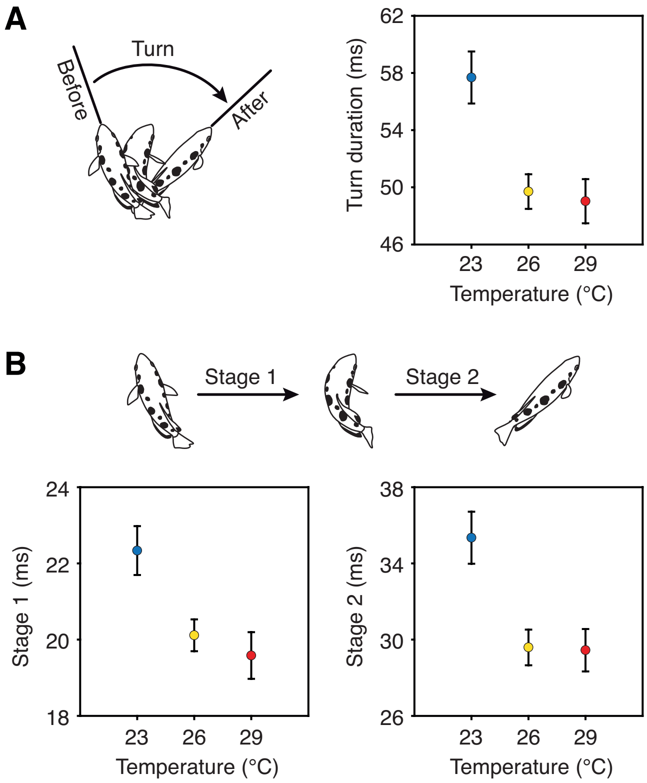

Fig. 3. Kinematic aspects of the C-starts are only partly temperature compensated. (A) Cooling below 26°C increased total duration of the predictive C-starts. (B) Splitting the turn into its two distinct stages, stage 1 (bending into a C-shape) and stage 2 (return flip), showed that the two stages are similarly affected by temperature. In A and B, durations are reported as means ± s.e.m. *N*=119, 195 and 115 C-starts analysed for 23, 26 and 29°C, respectively. Kinematic features were stable after a 1 week acclimation period to the experimental temperature. All durations were significantly larger at 23°C than at the two higher temperatures (Kruskal–Wallis: all tests *P*<0.001, Dunn's tests: *P*<0.05) but did not differ significantly between 26 and 29°C (Dunn's tests: *P*>0.05).

pattern also held for the two phases, stage 1 and stage 2, of the C-start manoeuvre (Fig. 3B). The duration of the initial bending phase (stage 1) was significantly longer at 23°C but constant at 26 and 29°C (Kruskal–Wallis: $H$=12.32, d.f.=2, $P$=0.002, Dunn’s test: $P$<0.05 for 23°C *versus* 26 and 29°C). Similarly, the subsequent rapid straightening phase (stage 2) was also significantly longer at 23°C but constant at the two higher temperatures (Kruskal–Wallis: $H$=14.96, $P$<0.001, d.f.=2, Dunn’s test: $P$<0.05 for 23°C *versus* 26 and 29°C). The increased total duration of the C-start at the lower temperature seemed to be accompanied by a change in the shape of the distribution of C-start duration. At 23°C, the distribution of total time was about 10 ms broader than at the higher temperatures (rank-based Brown–Forsythe: $P$=0.02), whereas no difference in the shape of the distributions was evident at 26 and 29°C. Cooling from 26 to 23°C thus increased the total duration of the manoeuvre from 46 to 56 ms (or about 3 ms °C$^{-1}$) and slightly broadened the distribution of turn duration. Acclimation to higher temperatures above 26°C seemed to leave C-start kinematics unchanged.

The duration of the two stages has previously been shown to be quite variable and related – among other factors – to the degree of turning (Wöhl and Schuster, 2007). It is therefore important to stress that turn sizes were equally distributed at the three temperatures (Kruskal–Wallis: $H$=0.03, d.f.=2, $P$>0.99; see Fig. 4B) so that the observed differences in acclimated turn duration are due to temperature and not to the fish making systematically smaller turns at higher temperatures.

### Precision of post-start alignment is unaffected by temperature

Already, the first tests after the brief acclimation period showed that the accuracy of the predictive starts was not at all affected by temperature. To account for the trial-to-trial variability in the way the target moved relative to the responding fish, we defined the error e as illustrated in Fig. 4A. Zero error means that the C-start leaves the fish perfectly aligned to the later point of impact, an error of –10 mm means that strictly following the initial alignment would lead the fish 10 mm past the landing point, in a direction towards the starting point of the prey. The error would be +10 mm if it was directed away from the point at which prey motion started. Histograms in Fig. 4A show that at all temperatures the errors were symmetrically distributed and always had a mean of zero (one-sample $t$-test: in all cases $P$>0.05). As reported in Table 2, this must already have been established at the end of the brief acclimation period, as we found no trend in error e over time. Moreover,

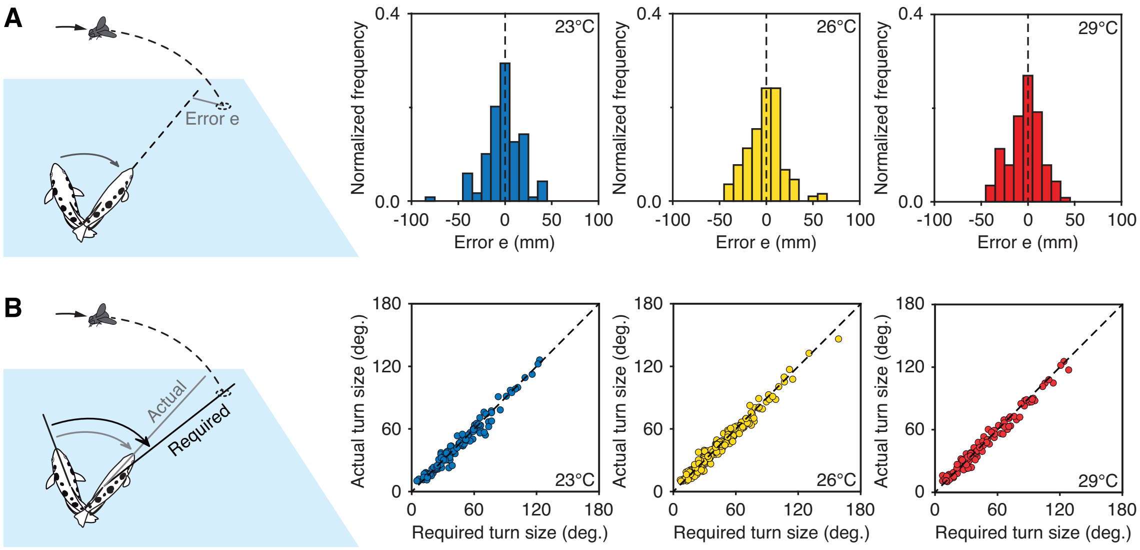

Fig. 4. The precision of aim after C-start is completely temperature compensated over the full angular range. (A) Histograms showing the distribution of errors in the initial alignment of the fish at the end of their C-starts. The accuracy of alignment was assayed by examining how close continued motion in this direction would bring the fish to the later impact point (error e). Note that target direction varied from trial to trial relative to position and orientation of the responding fish. Errors were not affected by temperature (ANOVA: $F_{2,426}=0.374$, $P=0.69$) and aim after C-start was right towards the future landing point at all temperatures (mean error e not significantly different from zero; one-sample $t$-tests: in all cases $P>0.05$). Also, the scatter around the perfect average alignment was not affected by temperature: standard deviations of the respective error distributions were not significantly different (Levene: $P=0.95$). Histograms are based on $N=119$, 195 and 115 C-starts at 23, 26 and 29°C, respectively, and are normalized so that each total frequency equals 1. Centre bin from –5 to +5 mm with bin widths of 10 mm. Zero error is indicated by dashed lines. (B) Plots of actual *versus* required aim after C-start shows that the turns were – at all temperatures – accurately set over the full range of required turning angles. The correlation was highly significant ($P<0.001$) at all temperatures and regression lines are not significantly different from those expected if actual turn size equalled the required turn size (dashed lines).

not only did the average start remain precisely aligned towards the later landing point but also the scatter around this aim was not affected by temperature: the standard deviations of the distributions obtained at different temperatures were not significantly different (Levene: $P=0.95$). Hence, both mean aim as well as scatter around that aim were fully temperature compensated after the brief acclimation period.

With changes in temperature of only 3°C, it is natural to ask whether the expected changes in the aim were not simply too small to be detectable. Given the high accuracy of the initial alignment (evident in Fig. 4A), which translates to a zero mean error angle with a standard deviation of no more than 4.3 deg, a typical standard error of the mean in our experiments was less than 0.4 deg. Hence, we should have been able to detect even slight effects of incomplete temperature compensation, with a resolution of 1 deg. It is also important to stress that the criteria described in Materials and methods ensured that we included only the most demanding starts and excluded cases in which precision would have been trivial to accomplish. Yet, the temperature compensation could have been achieved by restricting the angular range at which C-starts were launched. We therefore analysed the correlation of actual total turn size during the C-start manoeuvre with the amount of turning that would be required to be aligned to the later landing point. Would the fish be able to select an appropriate turning angle only in a restricted angular range? Fig. 4B shows that this is clearly not the case: C-starts came from a broad angular range at all temperatures and were equally accurate no matter what the required size of the turn was ($R^2$=0.97, 0.97, 0.98 for a temperature of 23, 26 and 29°C, respectively).

### The ability to compute post-start speed is unaffected by temperature

In addition to aligning the fish to where the prey is going to land, the C-start pushes the fish off with a speed that appears to be matched to distance and remaining time so that the fish will arrive shortly after the impact of its prey and at minimum travel cost (Wöhl and Schuster, 2006). Would the fish's ability to judge distance and timing be compromised after a change in temperature? This should in principle be detectable from an analysis of how the fish set their post-C-start take-off speed.

To establish such an approach we first examined whether the fish actually had acquired constant speed when stage 2 of their C-starts had ended and also whether they initially kept that speed (Fig. 5). To address this we took – for all starts at the three major temperatures – speed levels in four successive intervals of 10 ms duration that followed the end of stage 2. Fig. 5 reports the changes in speed between these intervals. Mean speed change between successive intervals was zero at all temperatures. Hence, take-off speed was attained in the first 10 ms subsequent to stage 2 and remained constant for the first 40 ms after take-off. It is thus safe to say that at all temperatures fish took off at a constant speed (whose absolute level, however, could be different from start to start so as to be matched to the novel conditions) and had attained this speed level immediately after the C-start (Fig. 5; one-sample *t*-test: in all cases $P$>0.05; repeated measures ANOVA: in all cases $P$>0.05).

To judge the fish's ability to infer distance and timing at the different temperatures and to set its take-off speed accordingly, we needed to find a range of speed levels that would be equally attainable at all temperatures. Cooling shifted the distribution of post-C-start take-off speed towards lower values (Fig. 6; ANOVA: $F_{2,417}$=13.20, $P$<0.001, Holm–Sidak test: all pairwise $P$<0.05) but even at 23°C the fish were able to reach top speed levels of over 15 fish lengths $s^{-1}$. Because the fish themselves commanded target motion it was crucial to check whether shooting power was similar at the three temperatures. For

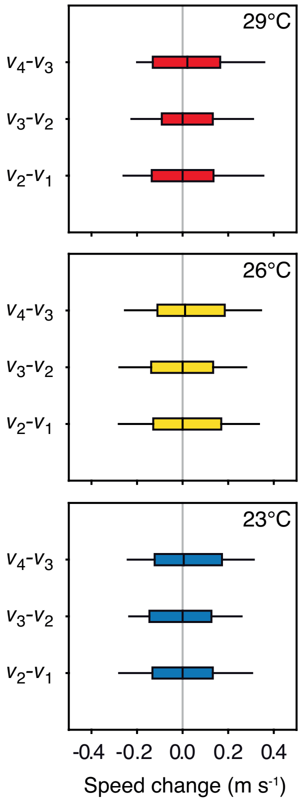

Fig. 5. Evidence that the C-starts resulted in stable initial take-off speed levels at all temperatures. Take-off speed, attained at the end of the C-start, was constant during the first 40 ms of the subsequent translation. For each response, speed values $v_1$, $v_2$, $v_3$ and $v_4$ were taken from the changes in position during the first, second, third and fourth 10 ms interval, respectively, of the fish's initial translatory motion. Boxplots show the changes in speed between the first two ($v_2$–$v_1$) and successive intervals. At all temperatures, mean changes were always not significantly different from zero (one-sample *t*-tests: in all cases $P>0.05$) and there was no significant difference among the intervals (repeated measures ANOVA: all tests $P>0.05$). $N$=117, 190 and 113 responses at 23, 26 and 29°C, respectively. Boundaries (whiskers) of boxplots show 25th/75th (10th/90th) percentiles, lines mark median. Grey lines show speed change of 0 m s$^{-1}$.

instance, a decline in shooting power at the lower temperature would result in a smaller mean distance travelled by the falling prey and this, in turn, would cause a shift in take-off speed towards smaller values. Direct measurement of the actual distances the dislodged prey items travelled showed that they were the same at all temperatures (Kruskal–Wallis: $H$=0.87, d.f.=2, $P$=0.65), thus showing that shooting power was unchanged. Moreover, the range of distances that the responding fish actually had to cover was not different at the three temperatures (Kruskal–Wallis: $H$=0.87, d.f.=2, $P$=0.65) and hence it can safely be concluded that the distribution of speed (Fig. 6) was also not confounded by the change in the mean distance the fish needed to cover, pointing to basic limitations of the fast-start motor system in the cold.

To analyse temperature compensation in the fish's ability to judge distance and time from the adjustment in take-off speed, we needed to select a range of speed levels that the fish could actually attain equally well at all temperatures. Based on Fig. 6 we selected the range up to 15 fish lengths s$^{-1}$ (on average 1.37 m s$^{-1}$) as one that would allow us to find out from the adjustment in take-off speed how well the fish had judged the situation.

We first asked whether take-off speed would better be predicted by the distance the fish had to cover to the point of prey impact or to this distance divided by the remaining time

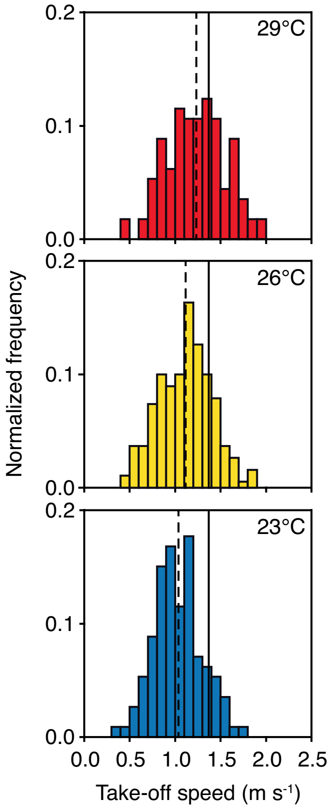

Fig. 6. The range of attainable speed depends on temperature. The distribution of initial speed values of starting fish was temperature dependent (ANOVA: $F_{2,417}$=13.20, $P$<0.001, Holm–Sidak test: all pairwise $P$<0.05). This must be accounted for when take-off speed is used to assay how temperature affects the fish's ability to judge distance and time. Speed levels from 0 to 15 lengths $s^{-1}$ (solid line) are attainable at all temperatures. Histograms are based on $N$=117, 190 and 113 responses at 23, 26 and 29°C, respectively, and normalized so that each total frequency equals 1. Binning starts at zero with bin widths of 0.1 m $s^{-1}$. Mean speed is indicated by dashed lines.

until impact (i.e. the 'virtual speed'). The analysis reported in Table 3 clearly shows that at all temperatures take-off speed was better predicted by virtual speed and not by distance alone. Fig. 7 therefore plots take-off speed *versus* virtual speed to assay the fish's judgement of distance and timing at the three experimental temperatures. Actual and virtual speed correlated equally well at all temperatures and no significant differences in slope ($P$=0.69) or intercept ($P$=0.06) of the regression lines could be detected (multivariate linear model: $R^2$=0.574, $F_{3,151}$=67.89; $P$<0.001). This suggests that the fish had perfectly temperature compensated their ability to set take-off speed (within the accessible range). This is also evident from a more direct way of measuring precision by analysing the distribution of the differences between actual speed and virtual speed. All distributions (not shown) had zero means and standard deviations (around 0.18 m $s^{-1}$) did not differ between temperatures (Levene: $P$=0.87).

The findings have two implications: first, in the cold, deficits in the motor system become apparent and maximum take-off speed declines; second, the 'computational' aspect of the problem – judging distance and timing, and setting take-off speed accordingly – is completely temperature compensated.

Table 3. Linear models show that 'virtual speed' describes the actual take-off speed attained at the end of the C-starts better than distance

| Temperature | Model | *k* | AICc | $D_i$ | $w_i$ |
|---|---|---|---|---|---|
| Temperature as fixed factor | **Virtual speed** | **5** | **-133.640** | **0** | **1.000** |
| | Distance | 5 | -88.908 | 43.732 | $3.190\times10^{-10}$ |
| 23°C | **Virtual speed** | **3** | **-23.291** | **0** | **0.912** |
| | Distance | 3 | -18.609 | 4.682 | 0.088 |
| 26°C | **Virtual speed** | **3** | **-74.109** | **0** | **1.000** |
| | Distance | 3 | -51.190 | 22.920 | $1.055\times10^{-5}$ |
| 29°C | **Virtual speed** | **3** | **-26.803** | **0** | **1.000** |
| | Distance | 3 | -10.889 | 15.914 | $3.501\times10^{-4}$ |

k, number of parameters in model; AICc, corrected Akaike information criterion; $D_i$, AICc–minAICc; $w_i$, Akaike weights. The best fitting model in each case is shown in bold.

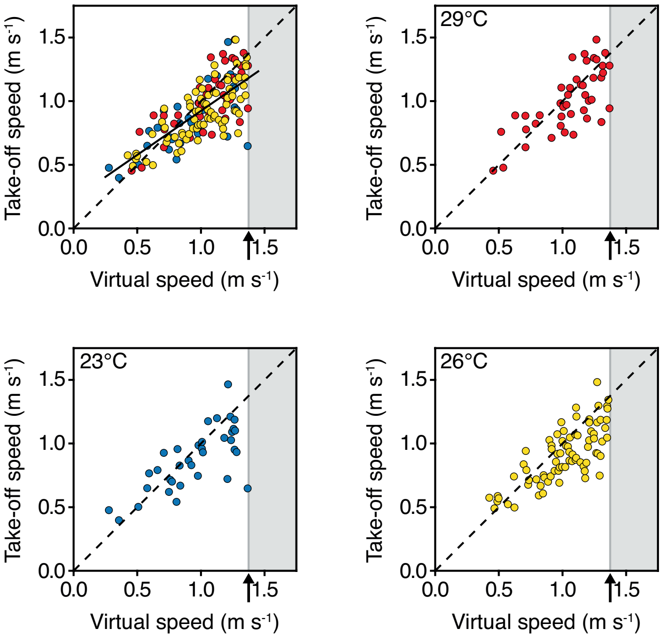

Fig. 7. Precision of setting initial speed is temperature compensated. At all temperatures the initial speed (take-off speed), attained immediately after the C-start, correlated best with 'virtual speed', i.e. the level of constant speed that would allow the fish to arrive simultaneously with the falling prey. To account for the decline in maximum speed with decreasing temperature, we compared the accuracy in setting initial speed only in the speed range that was accessible for the fish at all three temperatures. Based on the findings of Fig. 6, this was taken as 15 fish lengths $s^{-1}$ (arrow). $N$=35, 77 and 43 C-starts at 23, 26 and 29°C, respectively. Overall correlation is indicated in the top left plot (includes all data and regression line). Multivariate linear model: $R^2$=0.574, $F_{3,151}$=67.89; $P$<0.001; no difference in slope ($P$=0.69) and intercept ($P$=0.06). The three other plots show take-off speed *versus* required virtual speed for the three experimental temperatures (dashed lines indicate where speed was equal to virtual speed).

### Compensation in an extended range

Measurements made outside our standard temperature range illustrate how important the intrinsic motivational control was: both at 21°C (39 predictive starts in 39 trials) and at 32°C (19 predictive starts in 19 trials), responsiveness was 100%. That is, when the fish set their prey into motion they would also always produce C-starts. Furthermore, at both temperatures, the starts were accurately aimed, the mean errors were not different from zero (one-sample *t*-tests: in both cases $P>0.05$) and the standard deviations (0.02 m) were the same as those in the error distributions at all other temperatures (Levene: $P=0.999$). However, the motivation to hunt and to actually dislodge prey was largely affected by temperature. This is evident, for instance, from the mean number of dislodgements we were able to obtain within approximately 1 h of experimentation: 10 at 21°C, 20 at 23°C, 30 at 26°C, 35 at 29°C and fewer than 20 at 32°C.

## DISCUSSION

The fine-tuned archerfish predictive starts provide a sensitive assay of how well C-start manoeuvres can be buffered against temperature-induced changes in performance. In archerfish, the C-start must be matched to time and position of the point at which its ballistically falling prey will later impact and the effects of incomplete temperature compensation could readily and sensitively be detected. Moreover, the starts automatically offer an intrinsic control for matched motivation at different temperatures: because the fish first have to dislodge their prey, they elicit predictive C-starts only when they are actually motivated to hunt.

### Functional stability of fast starts

Our findings, obtained after a brief acclimation period, suggest that the functionality of the predictive C-starts is completely buffered against changes in environmental temperature. Whenever the fish are motivated to hunt, the release probability of their accurately aimed predictive starts is 100% throughout the full range of temperatures at which we observed hunting in the wild (about 23–30°C; S.S., unpublished data). Both the accuracy of the initial aim at the end of the C-start and the ability to set take-off speed according to estimated distance and timing appear to be completely temperature compensated. Note that we could have detected changes in either average alignment after the C-starts or the scatter around this average alignment with an accuracy of better than 1 deg. Moreover, the acclimation to a novel temperature must have been rapid, given that all properties were stable after our brief acclimation period of about 1 week. Our findings therefore point to a remarkable capacity of C-start networks to be ‘functionally stable’, i.e. to ensure precise functionality under changed operating conditions.

In goldfish, escape C-start acclimation allowed the animal to compensate only for the acute effects of cooling but not of warming up. An increase in escape probability and a decrease in directionality upon warming remained uncompensated even after 4 weeks (Szabo et al., 2008). Nevertheless, it is still possible that we presently largely underrate the degree of functional stability of escape C-starts. Stability of function may be masked by three major experimental difficulties with escapes. (i) Efficient escapes necessarily involve a random element that should not easily be detected by any observer. But without independent evidence on the acute levels of this random element only rather crude assays of functionality are possible. (ii) This implies that large changes in temperature are needed to see effects on directionality and these changes may not always be in the normal operating range of the animal. (iii) Escape C-starts share a further major difficulty with other assays of maximum locomotor capabilities – the problem of controlling motivation (e.g. Losos et al., 2002). While it seems natural to assume that the motivation to perform a life-saving start is always constant, this may not actually be true when alternatives exist: ‘freezing’ or simply suppressing the C-start could be alternative manoeuvres that might actually be better at lower temperatures. In the cold, doing nothing could be better than a C-start that is launched too late and that has insufficient power to move the fish rapidly enough out of the zone of danger.

Other C-start patterns of hunting fish would also allow both motivation to be controlled and accuracy to be assayed. Directed C-starts have been described, for instance, in fruit-catching Middle America machaca *Brycon guatemalensis* (Krupczynski and Schuster, 2008; Schuster, 2012). These stream-living fish respond to the falling motion of figs. The interesting part of their response is the way their starts accommodate the later amount of drift. The C-starts of *Brycon* rotate the fish in a direction that seems to automatically accommodate the amount of drift the fish will later face. We therefore hope that fast starts of these and other fish may provide useful systems to sensitively explore the more general capacity of the C-start network design to ensure functional stability after changes in temperature.

### Speed of acclimation

Because several weeks of acclimation were required in other fish (Webb, 1978; Sidell et al., 1973; Johnson and Bennett, 1995; Johnson et al., 1996; Szabo et al., 2008) and because incomplete compensation has been reported even after 8 weeks of acclimation (Webb, 1978), we had expected the acclimation of our fish to still be incomplete when tests started. We thus had hoped to detect at least small systematic changes during the first weeks. Surprisingly, we found no such changes (Table 2), even in those variables (latency and C-start duration) that were not temperature compensated. Thus, the acclimation appears to be accomplished in less than a week at the new temperature.

Rapid acclimation would seem to be a clear advantage for archerfish in the wild. In their natural mangrove habitats the interaction of tidal water movement with freshwater inflow causes temperature fluctuations at a given location. In addition, the irregular changes in water depth force the fish to move out from a good hunting ground to other suitable spots. Because these may differ in temperature from the previous one (for example because water depth or shading vary), archerfish probably have to regularly cope with changes in temperature. Yet, within the range of temperatures in which they hunt, their C-starts still need to be precise: incorrectly aimed starts mean overshooting prey impact and losing food while risking being spotted by predators. Hence, the ecological context would seem to strongly favour mechanisms that ensure rapid functionality after naturally occurring changes in operating temperature.

The situation in archerfish could be reminiscent of the extremely rapid acclimation found in marine intertidal invertebrates, for which the term ‘immediate compensation’ was suggested (e.g. Hazel and Prosser, 1974). In a brief interval following an acute massive temperature change, physiological properties of these animals are often unchanged, which frequently is described as the properties having effective $Q_{10}$ values close to unity. How would archerfish C-starts be affected by acute temperature changes? Would it be possible to discover ‘immediate compensation’ at least in the computations that underlie setting of C-start angle and take-off speed? Unfortunately, approaching the effects of acute changes on the behavioural performance is tricky: to critically and sensitively assay accuracy of the archerfish predictive start sampling of a few days is typically necessary. This is to ensure that starting conditions were really comparably challenging (see criteria introduced in Materials and methods). Based on the strong acute effects of temperature changes on all fish fast starts analysed so far (Webb, 1978; Beddow et al., 1995; Preuss and Faber, 2003;

Szabo et al., 2008), we think that an ‘immediate compensation’ of archerfish C-starts is not very likely. However, studies on acute effects are required to disclose the actual speed of acclimation and to dissect the involvement of various components (i.e. computations and motor power) of the archerfish C-start circuitry. At present it can only be said that the overall acclimation in the archerfish C-starts is remarkably fast – when compared with the several weeks needed in other fish (see above).

### Incomplete compensation of latency and C-start duration

While the C-start precision was temperature compensated, the latency of the C-start was not. Mean latency and variability of latency were increased at lower temperature (Fig. 2) and these effects did not acclimate any further. It may be tempting to speculate that latency increased to maintain function, i.e. to allow sampling of more information and thus to ensure maintained precision after cooling. While this holds true at a decreased visual contrast of prey motion relative to its background (Schlegel and Schuster, 2008) and at low light levels (P.K. and S.S., unpublished), it probably does not explain the changes in latency. First, latency changed with absolute temperature but not with changes from the initial temperature. Second, the amount of change was similar for latency and for the contraction phase (Stage 1), a relationship that we failed to see when latency was increased to maintain precision (Schlegel and Schuster, 2008) (P.K. and S.S., unpublished). Many factors – from visual transduction to the first contraction that makes the C-start visible – contribute to response latency and all of them are temperature dependent (see Lenz et al., 2005). So the most remarkable aspect of C-start latency appears to be that it showed so little variability and varied so consistently with temperature.

### Which factors are most important in mediating functional stability?

Fig. 8 shows that producing a fine-tuned C-start based on initial target motion is an integrative process with components that are affected by temperature in different ways: it requires the representation of information on the initial movement of prey (Rossel et al., 2002; Schlegel and Schuster, 2008), ‘computations’ to derive the required angle of C-start turning and required post-C-start take-off speed, and the capability of the body trunk muscles to work against resistance and fulfil what the circuits have computed should be done. Clearly, both the visual functions and the ‘computational’ aspects seem to be completely temperature compensated (Figs 4, 7) and compensation is achieved within no more than a week (Table 2).

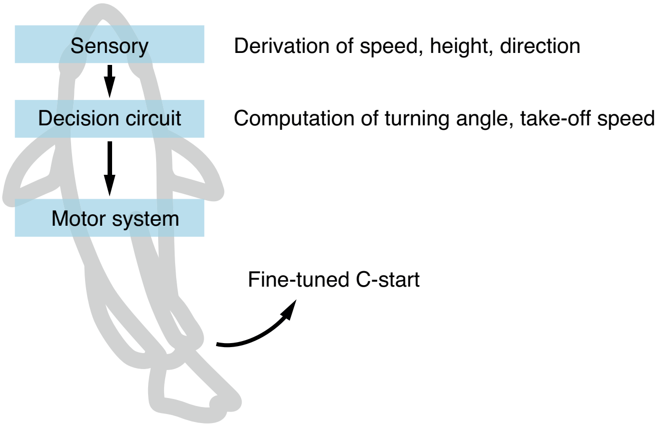

Fig. 8. Substrates that contribute to temperature compensation of the predictive start.

What would be interesting to explore further is the acclimation in archerfish trunk muscle. A huge body of literature (for reviews, see Bennett, 1985; Bennett, 1990; Rome, 1990) suggests that contraction speed does acclimate slowly – over the course of weeks – after acute changes in temperature. After cooling, an increase in the number of motor units can make up for the decline in contraction speed. Also, slow adjustments of the activity of myofibrillar ATPase activity – a major determinant of contraction speed – have been described (e.g. Bennett, 1990; Rome, 1990; Johnson and Bennett, 1995; Johnson et al., 1996). Therefore, what seems remarkable in the motor system of archerfish is that C-start kinematics were stable after only 1 week of acclimation. If acclimation in muscle occurred as in other fish, it should still be ongoing and detectable from changes in stage 1 or stage 2 duration during the testing phase, which was not the case. If acclimation was much slower than in other fish then it would still have been ongoing during the final experiments at 26°C that followed after a total of 5 weeks at 29°C. However, neither was a change in turn duration seen during the final tests at 26°C ($r$=–0.087, $P$=0.38) nor was turn duration different from that obtained in the first phase to which our fish had been acclimated for at least 1 year (Table 1). So either the archerfish muscles do not acclimate at all or they acclimate in less than about a week. The first option seems unlikely given that, 1 week after a 6°C change in temperature (from 23 to 29°C, see Materials and methods), the duration of the C-start (and of both its major phases) was stable and was equal to that at 26°C.

An alternative explanation, the use of thermally insensitive elastic recoil mechanisms (e.g. Anderson and Deban, 2010) seems to be at odds with our findings in Fig. 3. The ‘loading’ phase would correspond to stage 1 of the C-start and is affected by temperature in very much the same way as the subsequent straightening phase. Also, some sort of ‘buffering’ of the internal operating temperature of the trunk muscles is at odds with the shift in take-off speed in the cold (Fig. 6). Our findings thus raise the possibility that archerfish trunk musculature accommodates rapidly.

### Accessibility of archerfish Mauthner-associated network

Our findings suggest that the archerfish C-starts may help to bring a property of C-start circuitry into focus that remains hidden in C-start escapes: an exquisite stability of function against changes in operating conditions. The behavioural accessibility of the archerfish predictive C-starts comes packaged with neurophysiological accessibility of the Mauthner-associated hindbrain networks. Recent findings (P. Machnik, unpublished) show that archerfish do have Mauthner cells that are as easy to record from *in vivo* as they are in goldfish. The effects of temperature on the archerfish M-cells appear to parallel the findings in goldfish (Preuss and Faber, 2003; Szabo et al., 2008) and many properties of the archerfish M-cell are temperature sensitive. The accessibility of the archerfish C-start circuit may therefore facilitate our future understanding of the neuronal part played in functional stability, i.e. how function is stabilized when so many cellular and synaptic properties are strongly affected even by small changes in temperature.

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## AUTHOR CONTRIBUTIONS

S.S. and P.K. conceived the project, P.K. conducted the experiments, and S.S. and P.K. analysed the data and wrote the paper.

## COMPETING INTERESTS

No competing interests declared.

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# Meta-control ensures accuracy in high-speed decision-making

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**All decision-making appears to be bound by fundamental constraints[1,2,3,4,5,6,7,8,9] − such as tradeoffs between speed and accuracy − that govern decisions of acellular slime molds[1], social insects[2,3] and humans[4,5,6,10,11] in remarkably similar ways. However, it is unclear whether these apparently universal principles also constrain decisions that have specifically evolved to operate at highest possible speed with limited data. Analyzing one such decision – a complex multi-alternative decision that hunting archerfish must very rapidly make to secure their prey – we show that this decision manages to circumvent all the well-established fundamental constraints of decision-making[9,10]. Its accuracy is completely buffered against a demonstrably massive reduction in both input quality and time. Moreover, contrary to all predictions, the rate of declining the decision is not coupled to precision. The complete lack of any feedback that might have contributed to keeping the decisions accurate suggests a highly efficient meta-control mechanism that rapidly and selectively blocks only those decisions whose expected accuracy is below a threshold. We suggest that meta-control can be realized with simple networks that could significantly amend standard decision-making of humans and robots, allowing accurate decisions under adverse conditions instead of just blocking all decision-making when conditions get rough.**

The study of decision-making has culminated in a powerful framework that successfully predicts the coupling of accuracy, speed and confidence of decisions across an impressive range of systems and experimental designs[9,10]. For example, when the quality of sensory evidence (e.g. ‘stimulus strength’) deteriorates, the theory quantitatively predicts how this will decrease the probability $P_c$ of making a correct choice and how it will increase response time $\tau$ (Figure 1a). In the case of a decision made between two options, response time and probability of deciding correctly should be coupled according to

$$\tau = \frac{-2\alpha^2(2P_c - 1)}{\ln\left(\frac{1-P_c}{P_c}\right)} + \rho \qquad \text{(equation 1),}$$

with α, ρ scaling parameters (see Figure 1a and Extended Methods). The more recent discovery of fast decisions in olfaction and even in color detection[12,13,14] has been argued to also fit into the general framework when small amendments are made[15]. It thus seems to be presently agreed that we understand the general rules of decision-making[11].

Deciding when and when not to engage in a decision is another important feature of systems that are free to decline decisions (Figure 1b). Such systems can, in principle, avoid the penalty associated with too many false decisions, simply by declining decisions. When $P_c$ deteriorates, e.g. because stimulus strength decreases (Figure 1a), then the rate $P_{go}$ at which decisions are made should decrease up to a point beyond which no decisions are made. To predict this turn point, let g be the gain of a correct decision, f the investment made in each decision, so that the average net gain G is

$$G = P_{go}(gP_c - f) \qquad \text{(equation 2).}$$

It follows that no decisions should be triggered when $P_c < f/g$. From this simple treatment it may appear that $P_{go}$ should switch from 0 to 1 as soon as $P_c > f/g$. However, with errors in estimating $P_c$, $P_{go}$ will continuously increase with $P_c$ with maximal slope at $P_c = f/g$ (Figure 1b). Most importantly, beyond a certain level of accuracy all decisions should be declined.

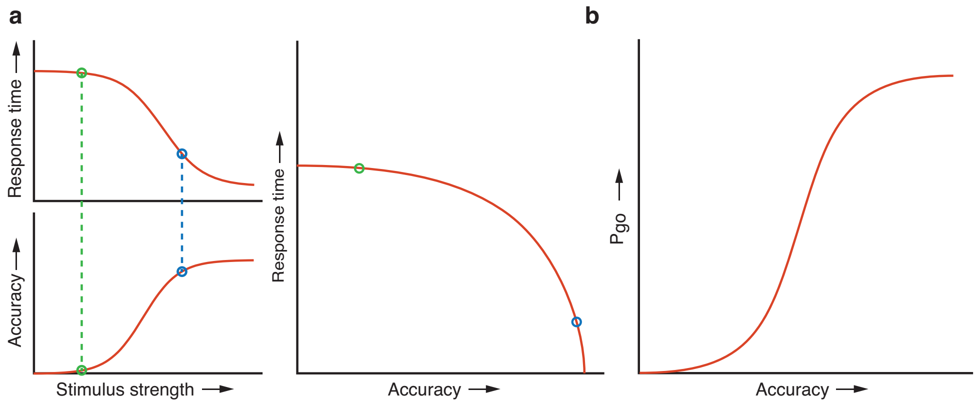

**Figure 1. Fundamental constraints in decision-making. a**, When the quality of the information required for the decision increases (increase in 'stimulus strength'), response time decreases and accuracy increases. As a result, changing stimulus strength causes correlated changes in response time and accuracy that can be quantitatively predicted. **b**, When decisions can be declined, changes in stimulus strength affect both probability $P_{go}$ to engage in a decision, and accuracy, again predicting a relation between $P_{go}$ and accuracy. We show here that high-speed decision-making efficiently circumvents such general constraints.

These remarkably general constraints of decision-making should in principle compromise decisions that are meaningful exclusively when made rapidly with limited data and time. However, such decisions often cannot always be blocked would thus often be erroneous, causing high costs of failure. The rapid decisions made by archerfish in the context of their unique hunting behavior[16,17] are one such example to challenge whether the general predictions also hold for highspeed decision-making. After one fish within a group has dislodged an aerial insect prey from its substrate with a well-targeted shot and when prey has just started its ballistic path towards the water surface, all fish rapidly select a motor program to be at the right spot in the right time and make the catch[18,19]. This decision is pointless if it is not made so that the fish is on its way before the impact of the falling prey alerts competitors. It is also pointless if it is not accurate: missing the impact point or overshooting it means losing the food while having become conspicuous to predators. Furthermore always declining the decision simply means no food.

Figure 2a introduces the design of our experiments and illustrates an important key feature of the multi-alternative fast-start decisions the competing fish make. Each responding group member is challenged with a randomly assigned configuration of initial movement (sensory information $S_i$) of a ballistically falling prey item and its task is to select an appropriate fast-start (motor response $M_i$) that turns the fish right towards the expected landing point. In the subsequent test, the configuration is changed randomly and the fish again has to make an adapted decision $M_{i+1}$, now for this new combination $S_{i+1}$. The most important feature of this design is that an evaluation of the error made in response $M_i$ would not help to correct motor response $M_{i+1}$. For example, if $M_i$ was 20 deg off toward the left side, $M_{i+1}$ cannot be corrected by now adding to the intended course 20 deg to the right. Furthermore, $P_c$ can be measured sensitively by monitoring the fish's error ($E_i$, $E_{i+1}$ in Fig. 2a) in aiming correctly, given the sensory information ($S_i$, $S_{i+1}$). The probability $P_c$ of correct choices is generally larger than 98% (see Supplementary Methods). Full experimental control over sensory information, as required in these experiments, is possible because motion cues are necessary and sufficient for the decision and a shooting context is not required[18].

To test the predictions illustrated in Figure 1 we shifted response time and responsiveness $P_{go}$ over wide ranges by adjusting input quality[9,10,11]. Our approach (Extended Data Figure 1) allowed dimming light while visual contrast was constant within the vast area from which responses came and the motivation of the fish to hunt was demonstrably unaffected. Reducing luminance did strongly affect the readiness of the fish to engage in the decision and allowed us to set $P_{go}$ anywhere from zero to one (Extended Data Figure 1c). It also allowed us to vary response time $\tau$ over a wide range from 40 to 228 ms (Extended Data Figure 1d). Hence, changing luminance provided the wide variations in $P_{go}$ and $\tau$ that we needed to test the predicted relations between speed and accuracy (Fig. 1a) as well as between responsiveness, $P_{go}$, and accuracy (Fig. 1b). Strikingly, however, within the full range of response time (Fig. 2b) as well as of probability $P_{go}$ (Fig. 2c), all decisions were completely buffered and perfectly accurate: Not only were speed and accuracy uncoupled, but top accuracy ($P_c > 0.98$) was maintained under all conditions although these demonstrably had strongly affected $P_{go}$.

We ran a number of analyses to critically assess these unexpected findings. First,

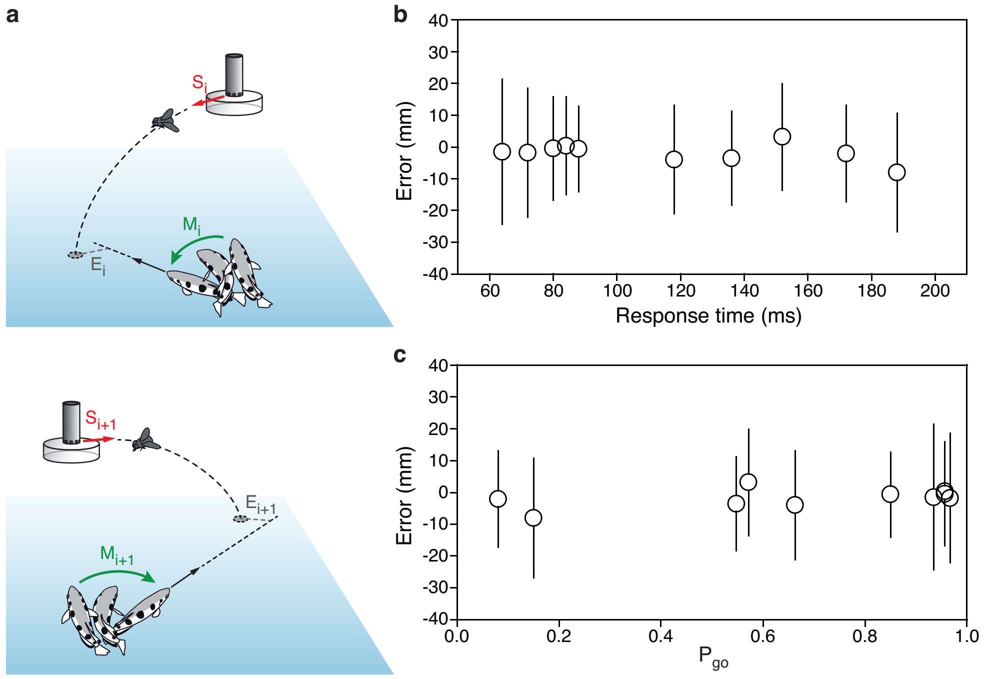

**Figure 2. Archerfish multi-alternative high-speed decisions do not obey the general constraints of decision-making. a**, Based on sensory information $S_i$ – a random combination of speed and direction in which a prey item is blown from a circular platform – archerfish select a rapid turn (motor response $M_i$) towards that point at the water surface at which the falling prey will later land. The accuracy of this motor decision can be sensitively assayed by the error ($E_i$) of the initial alignment after the turn. The combination of sensory information is randomly changed from one trial to the next (with $S_{i+1}$, $M_{i+1}$, $E_{i+1}$). Reliability of input, response time and response probability can all be experimentally controlled via changing luminance without affecting hunting motivation or the accuracy of assaying errors E (see Extended Data Figure 1). **b**, In contrast to the prediction (Fig. 1a), the decision shows no relation between speed and accuracy ($P = 0.23$, Pearson correlation between the error and response time). **c,** The decision also does not show ($P = 0.14$, Pearson correlation) the predicted relation between accuracy and probability $P_{go}$ of engaging in the decisions. **b and c**, Errors are mean ± s.d. as obtained under specific luminance levels (see Extended Data Figure 1) and translate to $P_c > 0.98$ throughout (all errors not significantly different from zero with equal variance). Total of $N = 564$ decisions.

motivation was constant in all experiments so that, for instance, $P_{go}$ did not simply change because of changes in the motivation of the fish to hunt. Motivation was checked regularly by placing prey on the bottom side of the experimental platform (Figure 2a). In all such tests ($N = 239$) at least one shot was fired – at all light levels – within less than 20 seconds after presenting prey and this shot always dislodged it. Second, dislodging prey without afterwards engaging in the C-start decision did cause a clear cost and meant loosing prey (Extended Data Figure 1e). Third, a closer look at the kinematics of the responses revealed that the fish had not maintained accuracy (Figure 2b, c) by altering the nature of the response elicited (Extended Data Figure 2b). Note also that the findings can not be attributed to a lack in complexity. Responses always came from all positions and orientations and were of equal accuracy over the full range (Extended Data Figure 2a). While most studies examine decisions between two[9,10,11] or four[20] alternatives, the archerfish's motor decision, in contrast, is made among at least 1000 alternatives[19] and yet was capable of fully maintaining accuracy ($P_c > 0.998$; see Supplementary Methods).

How did the fish then manage to exclusively elicit accurate decisions - even under adverse conditions? Did the fish at least initially produce the type of errors that standard decision-making theory would predict? If so, maintained precision would only be apparent and due to a capability of the fish to use feedback from these initial errors. Although both our random changes in the stimulus configuration and the regular shifts in illumination (after just a few trials) make this unlikely. A close look directly settles this point: The initial errors were not larger than those recorded later (Figure 3). The distribution of errors made in the initial tests at any new light level showed no dependence on trial number (all correlations $P > 0.1$). Furthermore, close inspection of all tests made on the very first day showed that the fish simply did not make any conspicuous errors even in their very first tests under adverse conditions (in which $P_{go}$ was below 15%). The errors in aim were just in the range observed under optimal conditions (dashed line) and were, moreover, not different from the first tests made under easy conditions (when $P_{go}$ was above 65%).

This shows, that the fish did not even initially make errors under adverse conditions. It also excludes mechanisms that would explain maintained accuracy from feedback that exploited the initial errors. In contrast, a control mechanism is at work that suppresses inadequate responses before they have actually been elicited. Our findings thus suggest a striking case of meta-control, in which an agent judges its potential success and then decides whether to engage or not to engage in the decision. Archerfish employ this

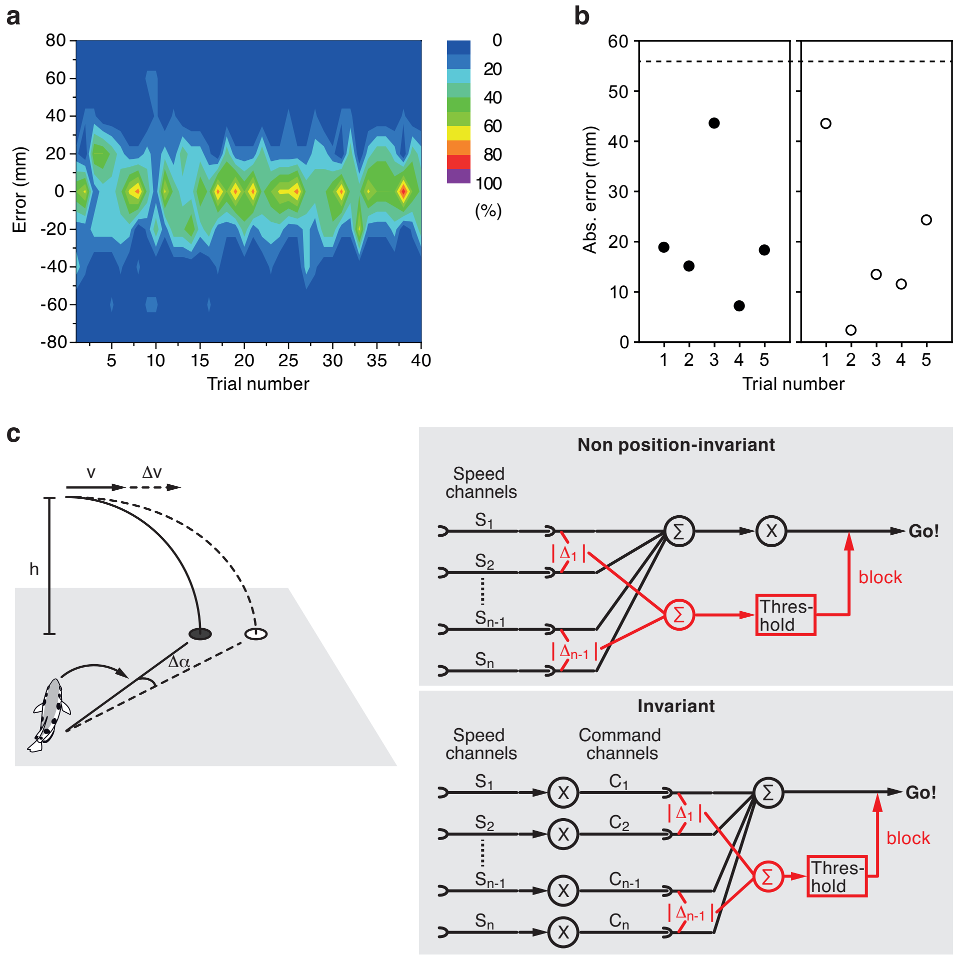

**Fig. 3. Meta-control requires no feedback to maintain full precision under adverse conditions.** **a**, Probability of an error of indicated size being made in the first, second etc. decision at any of the light levels tested shows that no errors occurred at any of the first trials and that error range stayed constant (correlation for each intensity $P > 0.1$). **b**, Absolute Error in the first five presentation each of this study in the dark (black circles; intensities 0.005 and 0.032 cd m$^{-2}$, $P_{go} < 15\%$ ) and under bright light (open circles; intensities 0.269, 0.472, 14.451 cd m$^{-2}$, $P_{go} > 65\%$) conditions directly shows that no errors occurred in the first exposition to adverse conditions (that heavily affected $P_{go}$). Dashed line shows range of errors in experiments under bright light conditions. **c**, Simple network architectures may be employed in meta-control. Sketch to explain how a set of speed coders could obtain measures of both average speed and scatter in speed estimate. If scatter signal is above threshold it blocks a motor command (calculated in circuit X) from being relayed to the motor system. While this architecture would not explain the complete orientation invariance of the decisions (Extended Data Figure 2a) it can be modified to operate not on the sensory data but on the computed motor control data. In this modified scheme, the motor response would be driven by the averaged motor command and would be blocked when the scatter in the independently computed motor commands is above threshold.

mechanism extremely efficiently: They suppress incorrect decisions even under difficult conditions in which they have to decline most decisions. Moreover, given that decisions can be elicited after 40 ms with no more information than what is available within this limited time[18], the judgement must also be surprisingly quick.

Although meta-control invokes aspects typically associated with high-level cognition[21,22], its neuronal implementation may not necessarily require complex circuitry.

Figure 3c shows a cartoon of the archerfish's task in which an error $\Delta v$ in judging speed v causes an error $\Delta\alpha$ in aim. A circuit arrangement that suppresses decisions with expectedly large $\Delta\alpha$ would simply consist of evaluating $\Delta v$, for instance by averaging the absolute values of differences of pairs of speed coders, thresholding this average and using an above-threshold signal to block the downstream passage of the motor command. Clearly, such a simple scheme cannot yet account for the positional and orientational invariance of the archerfish's response (Extended Data Figure 3a). For example, if initial motion is in the direction of the fish's length axis, then increases in $\Delta v$ would not cause variation in $\Delta\alpha$. The basic arrangement can, however, still be used and made invariant by shifting the evaluation of differences towards the motor output side. In this scheme, processing would operate on parallel channels that independently compute appropriate motor commands. Again, averaging these channels would yield the best motor command and averaging pairwise differences in the individual command signals would provide an error signal that could be used to block decisions that are unlikely to be accurate.

In summary we have shown that it is possible to beat the fundamental constraints of decision-making by employing meta-control. We argue that such meta-control mechanisms may not be difficult to implement and we predict that they will be found in many more decisions, specifically in decisions that have evolved to be made under pressure, cannot always be declined and have high costs of failure. Employing schemes for meta-control such as the one suggested in Figure 3c could help us to safely extend the range in which we are capable of making accurate decisions when conditions get rough.

## Methods Summary

Archerfish were confronted with randomly chosen initial movement patterns (speed, direction) of ballistically falling prey and had to select the appropriate motor pattern from a large number of alternatives. Accuracy, response time and probability $P_{go}$ of engaging in a decision were monitored using digital high-speed video. Our setup allowed us to systematically increase the difficulty of the decisions causing response time and $P_{go}$ to vary over wide ranges. This allowed us to test the predicted correlations between accuracy and $P_{go}$ as well as between response time and accuracy.

**Online Content** Additional Methods and Extended Data display items are available in the online version of the paper

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**Supplementary Information** is linked to the online version of the paper.

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**Author Contributions.** S.S. and P.K. conceived the project, P.K. conducted the experiments, and S.S. and P.K. analysed the data and wrote the paper.

**Competing Interests.** No competing interests declared.

## Methods

***Animals.*** Experiments were performed on a group of six archerfish, *Toxotes jaculatrix* Pallas 1767. The body lengths of the fish (from snout to caudal peduncle) ranged from 8.2 to 12.5 cm (10.2 ± 1.1 cm, mean ± s.d.). Fish were kept and all experiments were carried out in a large tank (1.0×1.0×0.6 m) filled to a height of 35 cm with brackish water (conductivity 3.5 mS $cm^{-1}$; temperature 26°C). An overall light regime of 12 h:12 h was maintained and experiments were started no earlier than 1 h after light onset (details below). All group members responded to dislodged prey, therefore, their responses were pooled.

***Experimental setup and protocol.*** The basic design is illustrated in Extended Data Fig. 1a, b. One dead fly (Calliphora sp.) at a time was blown from a transparent circular platform (acrylic glass, 50 mm in diameter), mounted 35 cm above the water surface in the center of the tank, so that the trajectories of falling flies on average lasted 267 ms. The center of the platform held a flexible tube (10 mm in diameter) with eight equally spaced air-valves (3 mm diameter each) out of which an air current could be directed at the fly placed on the 20 mm rim, which then left the platform and fell ballistically. The activation of the air current was not visible to the fish. Each of the eight air-valves was used in random order, so that both initial speed and direction of prey take-off could be varied randomly from trial to trial. To keep the fish motivated and attentive, trials were interspersed in which a fly was wetted and stuck on the lower side of the platform, so that the fish could shoot at and actively dislodge the fly.

Because critically assaying the decision required facing the fish with random combinations of initial motion of prey, the possible impact points lay in a wide range within the tank and responses came from the full area of one square meter of the tank. This required a setup in which we could dim the light in such a way that luminance and the contrast of the moving target with respect to its background was homogeneous over a wide, homogeneously illuminated, area. Furthermore, the setup had to allow varying luminance over a wide range while keeping contrast between prey and background constant. We achieved this by directing two LED spotlights (GLOBO 34100, 60×0.075W, GLOBO Handels GmbH, Austria) upwards onto a sheet that covered 2.0×2.0 m at a height of 1.5 m above the water surface. The reflected light from this background was the only source of visible light during the experiments. By placing three different Plexiglas® sheets with different transparencies (1%, 18%, 43%; 20×15 cm; Evonik Industries AG, Germany) in various combinations onto the LED spotlights, 11 luminance intensities were achieved in the range from 0.001 to 39.757 cd $m^{-2}$. The luminance of the background was measured on ten different spots to check the uniformity of luminance for each intensity level and averaged (LS-100, Konica Minolta Sensing Europe B.V., München, Germany). Note, with this design the resulting Michelson contrast between the falling fly and its background was constant and ranged from 0.96 to 0.98 for all luminance levels of this study.

We simulated sunrise and sunset by having the LED spotlights as the only light source 2.5 h in the morning and 2 h in the evening. In between, the normal room light was used. Experiments were conducted in the morning one hour after light onset of the spotlights. Up to four different luminance intensities were tested per day. The first luminance level tested each day was either the lowest level (70% of cases) or the highest (30% of cases) level, so as to check whether having spent more time in the dark or starting with success in the task would affect the results (which it did not). Afterwards, intensity was changed randomly after every 5 to 10 trials. After each change 5 (when changes were small) to 10 min (for large changes) were given for the fish to adapt to the new light level. Each experimental session comprised an average of 30 tests, 3.4 luminance levels and 4.6 level changes.

***Recording.*** We used digital high-speed video recording at 250 frames $s^{-1}$ (Fastcam MC2 Modell 500, PHOTRON LIMITED, West Wycombe, United Kingdom; lens Lensagon 4.4-11mm f/1.6, Lensation GmbH, Karlsruhe, Germany). The camera was mounted above the background sheet and viewed the water surface from a height of 1.5 m at a right angle through a hole in the sheet. For better recording two infrared spotlights (RAYMAX 300 PLATINUM AI 30, 850 nm, Raytec, Ashington, United Kingdom) were directed downward onto the water surface and a diffusor plate mounted below the tank was used to reflect the infrared light (Extended Data Figure 1a). The LEDs were selected because of the known cutoff in spectral absorbance of archerfish photoreceptors at around 700 nm[23]. Most importantly, however, our results clearly show that the fish could in no aspect of their performance profit from the IR light (see Results).

Because target and fish were at different distances from the camera, we corrected for the resulting metric distortions by projecting the falling prey onto the water surface, including the distortion in perspective that applied for the fly's current height level. Since the flies fell on a ballistic trajectory, the height level could simply be calculated from the time of falling, a procedure that has been used previously[18,24].

***Data analysis.*** The recordings were processed using ImageJ (developed at the National Institutes of Health) and custom-written software. All responses were C-starts as described earlier[18,25]. We evaluated C-start frequency (responsiveness $P_{go}$), response time, accuracy of the fish's rapid turn, and aspects of the C-start kinematics. C-start frequency was calculated for the trials of one session as the fraction of trials in which at least one fish showed a full C-start and take-off prior the fly's impact (with no requirement on directionality). Additionally we confirmed responsiveness $P_{go}$ by additionally considering the average number of fish that responded with a C-start and take-off prior the fly's impact (data not shown).

Our analysis explicitly excludes trivial cases that are not helpful in critically assaying the decision-making under dim light conditions. Because accuracy was measured before the prey's impact it could not have been affected by the fish's use of mechanosensory cues. Cases in which the fish could simply follow the prey's motion or could simply start in a straight direction without turning were excluded by requiring a minimum of 10 deg in both turn size and between the fish's chosen route and the projected horizontal trajectory of the fly. Also starts in which a conspecific blocked the direct path towards the landing point were excluded from the analysis unless stated otherwise.

Response time was calculated from the number of frames between the onset of prey movement and the initiation of the C-start. Similarly, we obtained the duration of the C-start and its two distinctive stages. The initial bending phase – stage 1 – was defined as the time from onset of the C-turn until the fish's body was maximally bent and had the shape of the letter C, and the straightening phase – stage 2 – as the time from maximum bend until the end of the turn leading to the subsequent take-off phase. Accuracy of the turn was assessed as follows: the error E of the aim of the C-start was taken at the end of stage 2 as the minimum distance a line in the initial direction had from the fly's later landing point. As in earlier papers[24], this distance was considered negative if the line intersected the projected path of the fly before the impact point or the projected position of the prey, respectively; otherwise, it was taken as positive.

Probability $P_c$ of the decisions being correct could sensitively be made from the analysis of errors E. A conservative estimate is working out the angular error ε and $P_c = 1 - ε/360$, which yielded $P_c > 0.98$. This underestimates $P_c$ because the fish tune their takeoff speed[26]. A better estimate would be $P_c = 1 - \pi E^2/A$, where A is the free area. Assuming A is half the area of the tank, the observed accuracy would translate to $P_c > 0.998$ (based on $N = 564$ starts).

Response time and C-start kinematics were assessed only in the fish that responded first but accuracy was assessed in all C-starts that were released. Finally, we also measured the time between impact of the fly and the arrival of the first fish (arrival time) at two luminance levels, one in a bright condition (14.451 cd $m^{-2}$) and one in the dark (0.001 cd $m^{-2}$).

***Statistical analysis.*** All tests were run using OriginPro (version 9.0, OriginLab 2012) and performed two-tailed with an alpha level of $P = 0.05$. In post hoc tests, the level of significance was examined using sequential step-down Bonferroni corrections. Normality of data was assayed using Shapiro–Wilk tests and additionally confirmed with Q–Q plots coupled with histograms. Equal variance was checked using Levene tests and rank-based modified Brown-Forsythe tests, respectively.

The relationship between luminance (*I*) and C-start frequency or response time, respectively, was fitted with a 'logistic' equation describing a sigmoid increase or decrease:

$$y = (A_1 - A_2)\left(1 + (I / I_0)^s\right)^{-1} + A_2 \qquad \text{(equation 3)},$$

with $A_1$ = initial value (lower boundary), $A_2$ = final value (upper boundary), $I_0$ = centre, s = sensitivity (power). Note that the fits are bound by minimum and maximum values but that these are derived from the data rather than set a priori. In case of parametric data (C-start frequency, mean number of fish responding) the fit was made based on mean values, otherwise (response time) median values were used.

Furthermore, as non-parametric tests (e.g. for C-turn properties and arrival time), we used Kruskal–Wallis one-way ANOVA on ranks, post hoc tested with Dunn's method and Mann-Whitney U-test. Parametric tests (for error in aim *versus* luminance levels) consisted of one-sample t-tests. Correlations were tested using Pearson correlation.

***Derivation of Equation 1.*** The derivation starts with the well-established relations for a choice with two alternatives (e.g. reference 10). Changes in stimulus intensity *I* affect both the probability $P_c$ (i.f. $= p$) of making a correct choice and the response time $\tau$. After rescaling $I$ via $u = kI$ (with k a constant) the dependencies can be cast in the simple form:

$$p = \frac{1}{1 + e^{-2\alpha u}} \tag{1}$$

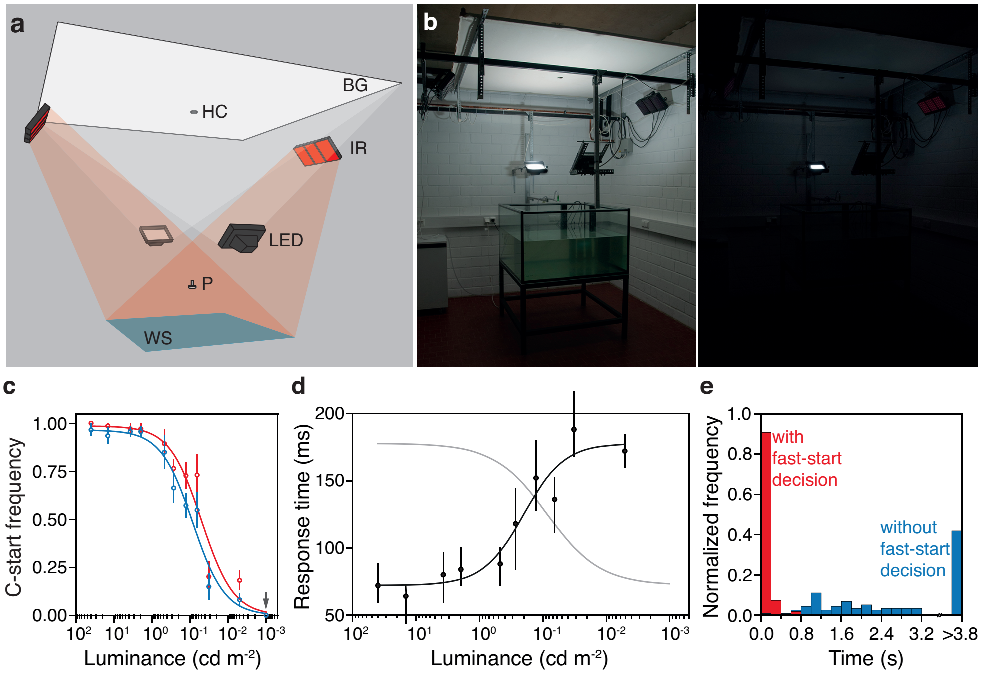
$$\tau = \frac{\alpha}{u}\tanh\left(\alpha u\right) + \rho \tag{2}$$

Now, eliminating $u$ from (1) it follows that $\alpha u = -\frac{1}{2}\ln(\frac{1}{p} - 1) = \ln\sqrt{\frac{p}{1-p}}$ .

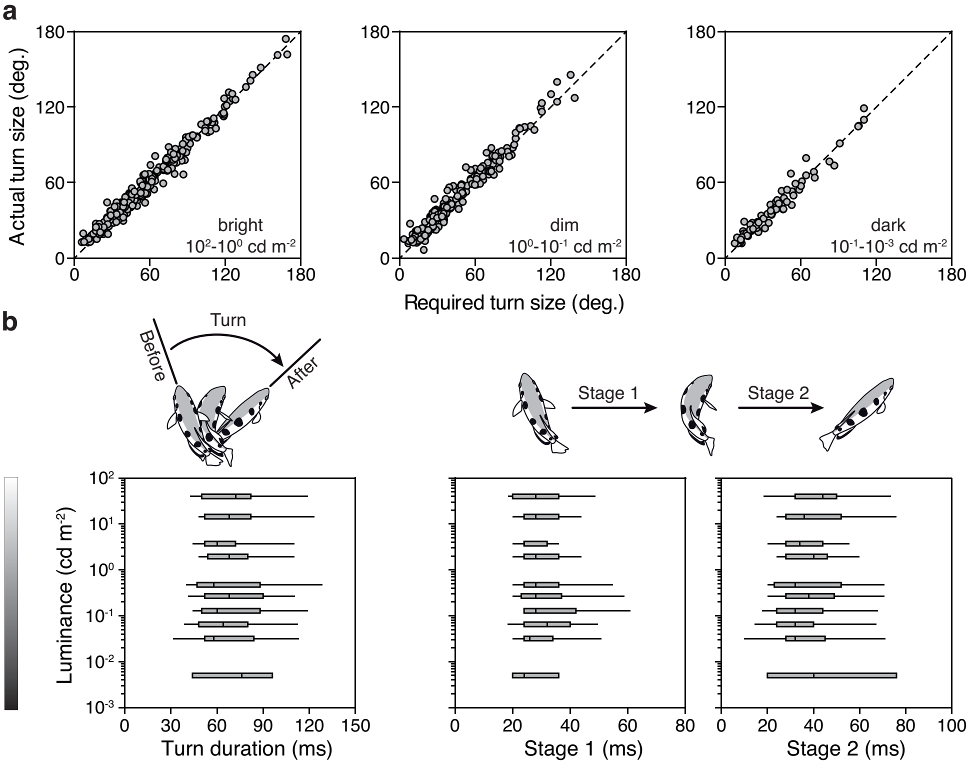
Hence $\tanh(\alpha u) = \frac{e^{+\alpha u} - e^{-\alpha u}}{e^{+\alpha u} + e^{-\alpha u}}$ = (S-1/S)/(S+1/S)=($S^2$-1)/($S^2$+1), where S = sqrt($p$/(1-$p$)),

so that

$$\tau - \rho = \frac{\alpha^2}{\ln\sqrt{\frac{p}{1-p}}}\frac{\frac{p}{1-p} - 1}{\frac{p}{1-p} + 1} = \frac{\alpha^2(2p-1)}{\ln\sqrt{\frac{p}{1-p}}} = \frac{-2\alpha^2(2p-1)}{\ln\left(\frac{1-p}{p}\right)} .$$



**Extended Data Figure 1. Varying response time and response probability $P_{go}$.** **a**, Setup used to assay how lowering illumination affects response time, accuracy and response probability of the high-speed decisions. Two LED spotlights (LED) were directed upward illuminating a background sheet (BG) at a height of 1.5 m above the water surface (WS) and ensured homogenous illumination. Acrylic sheets on top of the LED spotlights were used to change the luminance. A transparent circular platform (P) was mounted 35 cm above the water surface in the center of the tank. To achieve stable recording with a high-speed camera (HC) despite vast variations in illumination, two infrared spotlights (IR) were directed downward onto the water surface and a diffusor plate mounted below the tank was used to reflect the infrared light. The infrared light demonstrably did not affect the visual performance of the fish. **b**, Picture on the left illustrates a condition with bright luminance (BG: 14.451 cd $m^{-2}$), on the right is a scene in the dark (BG: 0.032 cd $m^{-2}$). **c**, Frequency (responsiveness) that at least one fish of the group made a predictive C-start decision (blue) or any C-start at all (regardless whether in time or too late; red) decreased rapidly with dimming and no C-starts were observed at $10^{-3}$ cd $m^{-2}$ (logistic regression for predictive starts, $R^2 = 0.96$, $F_{3,8} = 362.77$, $P < 0.001$, 50% response level at 0.09 cd $m^{-2}$). Frequencies are averaged per session (i.e. all trials of one day) and reported as means ± s.e.m. $N = 29(6)$, 54(9), 51(7), 51(7), 55(8), 76(10), 64(9), 65(8), 76(10), 55(9), 117(5) trials (sessions) analyzed from bright to dark. Arrow indicates that no C-starts were recorded in any fish at $10^{-3}$ cd $m^{-2}$, although the fish were able to detect and dislodge flies at this light level. **d**, Dimming increased response time of the fast-start decisions. Black line shows response time (logistic regression, $R^2 = 0.89$, $F_{4,6} = 176.18$, $P < 0.001$, 50% response level at 0.2 cd $m^{-2}$), grey line shows C-start frequency. Response times are reported as median ± quartiles. Numbers of C-starts analyzed: $N = 25$, 41, 40, 41, 42, 42, 33, 35, 12, and 7 (from bright to dark). **e**, The absence of fast-start decisions in the dark (0.001 cd $m^{-2}$, blue bars) strongly increases the time to catch (*U*-test: $U = 19.50$, $N_{bright} = 54$, $N_{dark} = 117$, $P < 0.001$). Histograms based on $N = 54$ and 117 responses at bright (14.451 cd $m^{-2}$) and dark light (0.001 cd $m^{-2}$), respectively, and normalized so that each total frequency equals 1. Binning starts at zero with bin widths of 0.2 s.



**Extended Data Figure 2. Controls to show that the complexity of the decision was not affected by changing illumination. a**, Plots of actual *versus* required aim after C-start show that the turns were – at all light levels – accurately set over the full range of required turning angles. This shows that constant accuracy did not result from the fish effectively simplifying the task. At all light levels correlations were highly significant ($R^2 = 0.99$, 0.98, 0.98 for bright, dim and dark conditions, respectively; $P < 0.001$) and regression lines are not significantly different from those expected if actual turn size equaled the required turn size (dashed lines). $N = 216$, 277, 71 starts analyzed for bright, dim, dark conditions. **b**, Maintained accuracy is not achieved by changes in the motor program. Dimming the light did not increase the total duration of the predictive C-starts (Kruskal-Wallis: $H = 4.01$, d.f. = 9, $P = 0.91$). Furthermore, splitting the turn into its two distinct stages, showed that neither stage 1 (bending into a C-shape; Kruskal-Wallis: $H = 7.84$, d.f. = 9, $P = 0.55$), nor stage 2 (return flip; Kruskal-Wallis: $H = 8.08$, d.f. = 9, $P = 0.53$) were affected. Boundaries (whiskers) of boxplots show 25th/75th (10th/90th) percentiles, lines mark median. $N = 25$, 41, 40, 41, 42, 42, 33, 35, 12, and 7 starts analyzed from bright to dark.

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## Report

# Fruit-catching fish tune their fast starts to compensate for drift

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## Summary

Numerous animal navigators are not simply at the mercy of winds and currents but cope with drift to reach their goals [1–7]. Here, we report how a fruit-catching Costa Rican fish combines an analysis of aerial motion with a novel way of compensating for drift to optimize its catching success. In the field, schools of this riverine fish never waited until a falling fruit actually landed in the stream. Rather, the fish responded to visual motion and started early to arrive on time at the spot where their food would land. To be successful with their early starts, the fish must cope with the strong relative drift that arises, because the fish, but not their airborne target, experience strong flow on their way toward the fruit's landing point. Surprisingly, the fish solve this problem right at the beginning – by turning rapidly and taking an initial aim that is already optimally adapted to the prevailing drift, so as to lead them straight to their food. Fruit-catching fish thus provide a stunning case of how rapidly animals can generate drift-compensating trajectories in their everyday local lives.

## Results and Discussion

Ripe fig trees can release all their fruit within a few days [8], so it pays for the large adults of the fruit-eating fish *Brycon guatemalensis* to wait below them. To mimic this situation, we released one fruit at a time from a tall bridge at the La Selva biological station (Costa Rica) into random positions in an area in the center of the stream (Figure 1A), giving the fish no a priori clue when and where the fruit would drop. The waiting fish stood stationary beneath the water surface, working head on against the stream. The school never waited for the impact of the fruit but responded much earlier, to the motion of the fruit. A typical scene is shown in Movie S1, available online. While the falling fruit was still up in the air (Figure 1B), the fish initiated a rapid turn and then started to move in the direction they faced at the end of the turn (their "initial aim"). In the turning phase, the fish first bent their bodies into a typical C shape (Figure 1C) and then straightened it into the direction of the subsequent take-off. On the basis of their duration, estimated average turning speed, and linear acceleration (Figure S1), the starts appear to be typical C-type fast starts, such as those commonly found in the rapid Mauthner cell-initiated escapes of teleost fish [9, 10] but also in the precisely aimed predictive starts of archerfish [11].

Interestingly, the fish turned and started not in broadly scattered directions but approximately toward the future point of impact. Actual turn sizes correlated significantly with those required for a direct alignment to the future point of impact ($r^2 = 0.77$, $p < 0.001$; Figure 1D). However, the match was not perfect: The actual turns tended to be smaller than required, and their regression line (red line in Figure 1D) differed systematically both in slope ($p < 0.001$) and in the offset ($p < 0.05$) from that required for a direct alignment (black line in Figure 1D). The nature of this apparent "deficiency" in the fish's aim will become clear later.

The early starts were not unspecifically triggered by any form of aerial motion, but required a downward component. In a series of experiments, fruit mimics were either upwardly or downwardly accelerated, with the same acceleration of $g = 9.81\ \mathrm{ms}^{-2}$ from a common initial height of 2 m above the water. Whereas downward acceleration readily triggered the normal response, and although the fish were highly motivated and responded well in interspersed controls – with real fruits released normally into the water from a standard height – the upward acceleration completely failed to elicit any response (Fisher's exact test, $p < 0.001$; Figure 1E; Movie S2).

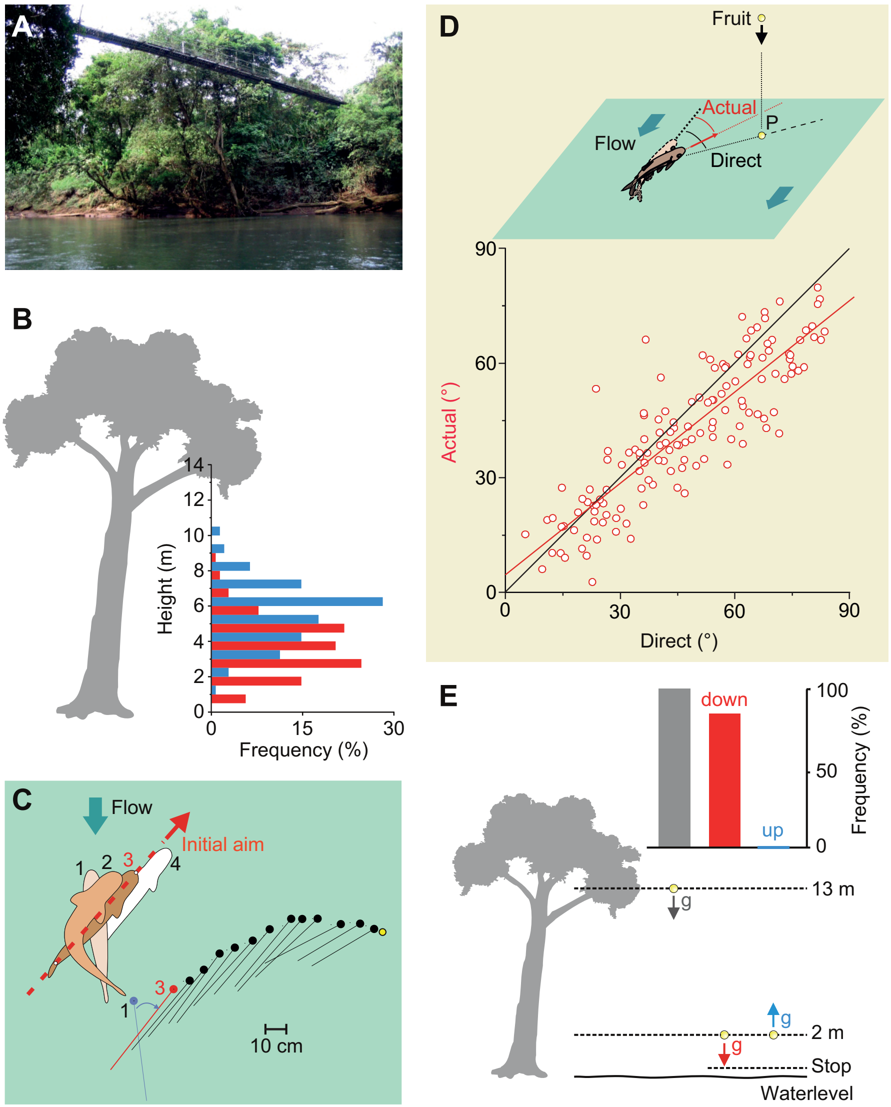

Figure 1. Fruit-Catching Fish Evaluate Aerial Motion to Initiate an Early Fast Start

(A) Stream at La Selva biological station in Costa Rica, with bridge from which fruits were released to a school of large fruit-catching *Brycon guatemalensis*. (B) Although the fish had no prior clue when and where a fruit was released, they were able to initiate (blue) an appropriate fast start and to take off (red) when the falling fruit was still at the indicated height above the water surface (n = 142 responses, bin width 1 m). (C) The start: Four silhouettes show the fish (1) immediately before its start (640 ms before the fruit's impact), (2) 80 ms later, when the body is bent into a typical C shape, (3) a further 60 ms later, when propulsion starts, the time at which we assessed the fish's initial aim (red arrow), and (4) after the first 60 ms of the fish's path. The subsequent path of the fish (head and length axis indicated every 40 ms by dot and line, respectively) is shown below. The fish arrived simultaneously (20 ms after final drawing) with its food at the impact point (yellow). This particular start was chosen to show how long even a completely erroneous initial aim is kept. (D) The initial aim is not random but is related to the later point of impact. Plot of actual turn size (“actual”) versus turn required for alignment to the food's later landing point (“direct”). The correlation is highly significant ($p < 0.001$) but the regression line (red) differed systematically from the prediction for direct alignment (black line). (E) The early starts are specifically triggered by downward motion. Experiments in which fruit mimics were accelerated with $g = 9.81\ \mathrm{ms}^{-2}$ from a 2 m initial height, either upwardly (n = 9) or downwardly (n = 19), are shown. Because the mimics did not land in the stream (“Stop”), we interspersed controls, in which real fruits (n = 28) were released normally, so as to keep and probe the motivation of the fish.

The motion-induced turns appear to be executed in an “open loop” mode. This is suggested by experiments in which fruit mimics were released from a low initial height of 2 m. Usually, they fell freely to the water and then responding fish were rewarded with a real fruit thrown into the water by the experimenter. These tests served as controls ($n = 63$ responses) and also served to keep the fish motivated. Interspersed were, however, a few tests (yielding $n = 8$ responses) in which a filament swung the mimic backward after 1.8 m of free falling, often in the midst of the fish’s rapid turn (Figure S4). Interestingly, this drastic change in the trajectory altered neither turn duration (control versus stop, Mann-Whitney U test: $p = 0.08$) nor its relation to turn size ($r = 0.76$, compared to $r = 0.74$ for the controls, $p = 0.93$, Fisher’s r-to-z transformation). Furthermore, the correlation between the actual turn size and that required for an aim toward the (real or predicted) landing point did not differ ($p = 0.15$, Fisher’s r-to-z transformation) between the control trials ($r = 0.97$) and trials in which the fruit’s trajectory was changed ($r = 0.89$). This, together with our finding that initial speed and direction were constant – even in completely erroneous starts (Figure 1C) – suggests that the motor program is already set before the beginning of the turn.

Monitoring aerial motion and starting early is only helpful when the fish solve an additional problem: that of severe relative drift. While the fruit falls freely in air, the fish must make its way toward the later meeting point through rapidly flowing water. Given that fruits were typically caught as soon as (less than 20 ms) they landed on the water, coping with the relative drift in the preimpact time is imperative. Figure 2A illustrates a way in which the fish could do this. If they tried to aim directly at the later landing point, they would need to continuously steer, leading to curved trajectories. Accounting for the flow would allow the fish to be faster. In a simple passage with constant travel speed through spatially and temporally constant flow, the fish could select an optimal upstream heading “error” relative to the beeline that – when kept to – would lead straight to the target. In the two cases shown for illustration in Figure 2B, doing this correctly would speed up travel time by at least 3% and 7%, respectively, which might be decisive in light of the heavy competition.

Two factors in our experimental situation make it straightforward to test whether the fish actually did start optimally. First, flow was uniform within our experimental area. Second, the fish started both with constant speed (Figure S2) and with constant orientation (Figure S3). We analyzed 142 starts made from various initial positions with respect to the

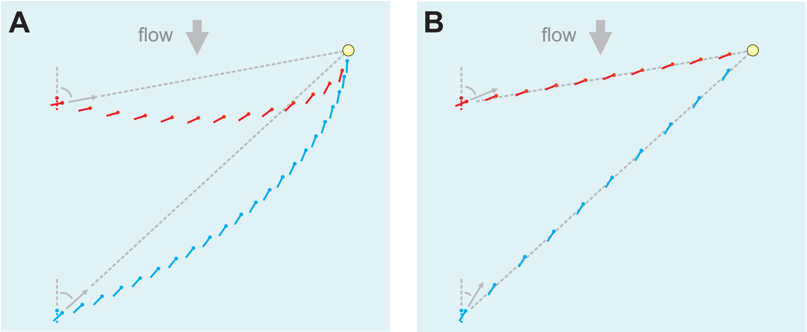

Figure 2. Strategies of Compensating for Relative Drift
While the fruit falls in air, the fish must move through rapidly flowing water. The effect of the resulting relative drift is illustrated with the trajectories of two hypothetical navigators when flow speed (gray arrows) is great, about 40% of the navigator's speed. Successive position and orientation are shown, with the dots indicating the head. In (A), the strategy is to keep a stable course toward the later landing point (yellow) of a falling fruit. In (B), the fish aim not directly toward their goal but choose an upstream correction that is matched to their start position, as well as to the prevailing flow, so as to lead them straight toward their target.

fruit's later landing point (Figure 3E). For each start, we measured the fish's prestart upstream ($\Delta y$) and lateral ($\Delta x$) distances (Figure 3A) and calculated the direct and optimal heading directions predicted from the fish's initial position. This yielded two errors, $E_\delta$ and $E_\omega$, that the fish's actual initial aim had with respect to the two predictions (Figure 3A). In the reference frame that we used for this analysis, the predicted directions for the given start were

$$\delta = \arctan D \tag{1}$$

for the direct aim, and

$$\omega = \pi / 2 - \arccos\left(\frac{k}{\sqrt{1+D^2}}\right) + \arctan D \tag{2}$$

for the optimal aim, where $D = \Delta y / \Delta x$ and $k$ is the relative flow speed.

Should the fish start optimally, their initial aims should scatter around the predicted optimal directions. In other words, the average of error $E_\omega$ should be zero, whereas at the same time, the average of $E_\delta$ should be systematically offset from zero. Conversely, if the fish's strategy was to aim directly, then the average of error $E_\delta$ should be zero and errors $E_\omega$ would be systematically offset from zero. Should the averages of $E_\delta$ and $E_\omega$ not differ, then our data would not allow us to decide which strategy the fish were using. The analysis shows clearly that the average of $E_\delta$ systematically deviated from zero (t test, $p < 0.001$). In contrast, the average deviation of $E_\omega$ from the predicted optimal initial aim was zero (t test, difference from zero $p = 0.38$; Figure 3B) and was also significantly different from

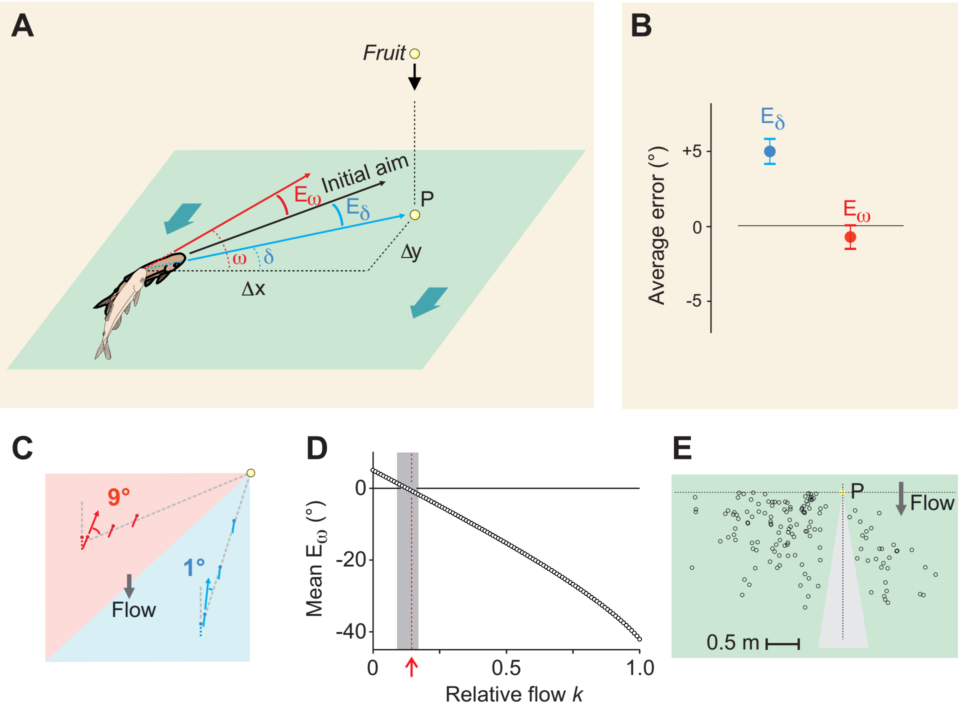

Figure 3. Fruit-Catching Fish Optimally Adapt their Fast Starts to Drift

(A) Analysis of the initial aim. Based on distances $\Delta x$ and $\Delta y$, the predicted heading directions (red and blue, oriented with angles $\omega$ and $\delta$ relative to the $x$ axis) were calculated for derivation of the errors, $E_\delta$ and $E_\omega$, that the fish's actual initial aim had with respect to these two predictions. A positive sign was given when the actual aim was more upstream than that predicted. In the case illustrated, the error $E_\delta$ would be positive and $E_\omega$ would be negative. The calculation used our estimate of an effective relative flow speed of $k = 0.14$ (see Supplemental Data). (B) The averages of errors $E_\delta$ and $E_\omega$, determined from 142 starts, show clearly that the fish did not minimize their error to the direct alignment but to the predicted optimally drift-corrected aim. (C) The drift correction is not fixed but is adjusted to the fish's initial position: Fish that were located laterally (region marked red) from the later impact point applied larger drift corrections than did fish located downstream (region marked blue). Numbers denote the average drift correction in the two regions ($n = 75$ lateral, SEM = 1.1°; $n = 67$ downstream, SEM = 1.1°). (D) Extending the analysis of (B) to other levels of flow speed, k. The average of error $E_\omega$ in all starts is shown when the predicted optimal heading directions are calculated for the indicated flow-speed levels, k. The arrow indicates $k = 0.14$. The range in which the error did not significantly deviate from zero (t test, $p > 0.05$) is shown by the gray bar. (E) The prestart positions relative to the fruit's landing point in our data set of $n = 142$ starts analyzed in (B)–(D). We use a common reference frame, with the impact point (P) always at the origin and the flow in the $-y$ direction. Actual impact points were scattered over at least 7 $m^2$. Responses in the gray area did not enter the analysis.

the error $E_\delta$ (t test, $p < 0.001$). The fish had, thus, turned and started so as to optimally account for the prevailing drift.

Did the fish start correctly – on average – by simply applying the same upstream correction regardless of where they started? This view can clearly be ruled out: When the fruit's later impact point was not directly upstream in front of them, the fish used significantly (t test, $p < 0.001$) larger drift corrections in their initial aims compared to those used when the impact point lay almost directly upstream before them (Figure 3C).

Would the drift correction also be adaptive in other than the prevailing flow regimes? Interestingly, this is not the case (Figure 3D). Their feeding ecology [8, 12] requires the fish to frequently move to wherever a tree with ripe fruit is found, where the flow pattern can be very different from that under the previous tree. This raises the interesting possibility that the fish might be able to adapt their drift correction to different flow regimes, but whether and on what basis they do this is completely open at present. Waiting fruit-catching fish appear to be able to choose to apply or not to apply a drift correction when they aim at food. This is suggested by an analysis of the paths taken by initially very distant fish 200 ms after their food had landed. At this time, the fruit experiences approximately the same drift as the fish does, and a drift correction would not be needed. Indeed, the fish then aimed straight at the target's actual position (average deviation from predicted direct aim 1.3°, not significantly different from zero, t test, $p = 0.10$, $n = 66$).

The major surprise of this study is that the fish efficiently dispose of drift right before it becomes a problem: by already starting correctly. Because the fish's initial aim is already correct and based on information sampled before they initiate their turns, sensory feedback cannot play a decisive role. This is not so in other navigators with more leisure. Bees, for instance, can arrive at a food source even in strong crosswind [2, 13]. To achieve this, their initial movement probes the direction in which the image of the surroundings moves over the eye. On the basis of this information, they would then adjust their movement so as to make the retinal image of the environment shift in the direction marked by the dance of another bee. In our hurried fish, the motor program must be ready, or ''planned,'' before the start is triggered. The fish can choose to adjust their visually driven C-type fast starts by adding a correction, $\Delta = \omega - \delta$, to their turn size, $\Phi$, that – on average – is optimally matched to drift. Our data suffice to say that the correction is not constant but do not allow us to conclude that the correction follows in detail the predicted relation $\Delta = \pi / 2 - \arccos\left(\frac{k}{\sqrt{1+D^2}}\right)$. The fish might, for instance, solve the problem by adding an extra turn size that is a set fraction of $\Phi$.

Our findings add another example [14, 15] in which a task that would qualify being labeled as "motor planning" can be carried out rapidly, probably on the basis of the fish's fast-start circuitry [10, 11, 14]. Dealing with drift is a major challenge in long-distance migrations, in which compensatory mechanisms are studied the most [1–7]. The efficient solution of fruit-catching fish reminds us that drift compensation is a skill that is probably found in a large variety of mechanisms in the local everyday lives of many species. The study of such local mechanisms [16] might be instrumental for understanding the ease at which the outstanding skills of so many diverse groups of long-distance migrators have evolved [5].

## Experimental Procedures

All experiments were carried out in the field during the March–May dry season at the La Selva biological station (Costa Rica). One fruit at a time was released from a tall bridge (13.5 m above the water) into a randomly chosen spot within the experimental area in the center of the Rio Puerto Viejo. An experimental area about 15 m from the shores (water depth approximately 1 m) was chosen so that constant and spatially homogeneous flow speed could be ensured (as inferred from the absence of rotation in floating leaves). The responses were recorded on a camcorder (Sony DCR-TRV270E) that was mounted on the bridge to look orthogonally unto an area in the stream sized 4.6 m × 3.4 m. The actual height of the camera above the water level was measured for each session and was used for spatial calibration of the recordings. The recordings were digitized (Magix Video 2005) and deinterlaced (Avisynth 2.5 and Virtual Dub 1.6.8) to provide 50 half-frames/s for subsequent quantitative analysis (Image J 1.34n).

Experiments were done early in the morning when recording conditions were best. Fruit were standardized pieces of banana, cut to 4 cm in length (to match the size of figs) and released so that the fish had no clue about where and when a fruit was released. The first fruits released early in the morning attracted schools of 15 to 35 adult fish to the experimental site. The total length of each fish (45 cm ± 8 cm, mean ± SD, n = 142) that contributed to the data set in the analysis of Figure 3 was directly measured from the video frame that immediately preceded its response.

## Analysis of the Initial Aim

We analyzed the initial aims taken by fish that were located downstream of the target (Figure 3E). For the exclusion of potential influences that the responses of other school members might have on the aims taken, only the first fish that responded in each trial was analyzed. The aim of a second (and third) fish that responded equally fast was determined (in 27 and 11 of the 142 responses, respectively) when at least 1 m separated the fish and when turns differed by at least 30°. Furthermore, to ensure that the fish could use exclusively visual information for choosing its initial aim and not additional mechanosensory information, we only analyzed the initial aim when the fish took off at least 0.04 s before the fruit's impact. Two additional important criteria were that the fish were not blocked in their initial takeoff by other fish and that they had to turn by at least 10° from their initial orientation against the stream (so that response onset could easily be detected). Timing could be reliably quantified by the sole time stamp available, the fruit's impact time, because fruit fell ballistically, with negligible friction throughout the height range of our experiments. The actual height of the fruit, a time $\Delta t$ before impact, could therefore be derived from

$$h = h_0 - \frac{g}{2}(T - \Delta t)^2 , \qquad (3)$$

in which $h_0$ is the initial height of the fruit and T is the total time from release to impact.

### Experiments on the Specificity of the Motion Trigger

In the tests on the specificity of the motion trigger, three artificial fruits (wooden spheres 4 cm in diameter, attached to thin nylon filaments and painted yellow to match the bananas) were hung from the bridge. They were stationary at a common height and any of them could at any time be accelerated either downwardly or upwardly. A low initial height of 2 m above the water was chosen so that best visibility for both motion directions was ensured. An upward-pointing acceleration with strength g was produced by connection of the artificial fruit to a distant stone via a fishing line that ran over pulleys. Releasing the stone far away from the experimental site started the upwardly accelerated motion (see Movie S2). With downward acceleration, the artificial fruit was stopped shortly (20 cm) before it hit the water surface. This ensured that – symmetrically – no fruit would hit the water after both upward and downward acceleration. To keep the fish motivated during the tests and to probe their motivation, controls were interspersed, in which normal fruits were released into the water from the bridge as in all other experiments.

### Predicting the Optimal Aim

Consider the situation of Figure 3A with the fish originally located a distance $\Delta y$ downstream and $\Delta x$ to the side from the later impact point of a falling fruit. Let $v_F$ be the effective speed of flow, $v_W$ the fish's speed relative to the water, and $\beta$ the take-off angle measured from the *x* axis; then, the components of the fish's speed relative to the shore are

$$\begin{aligned} v_x &= v_w \cos\beta \\ v_y &= v_w \sin\beta - v_F \end{aligned} \quad . \tag{4}$$

A possibility of eliminating $v_W$ arises when the fish covers distances Δx and Δy right in the time T that remains from the onset of the fish's start until the impact of its food. This would require an initial aim of

$$\beta = \arctan\left(\frac{\Delta y + T v_F}{\Delta x}\right) . \tag{5}$$

But the underlying assumption is clearly at odds with our direct analysis of the fish's take-off speed. Rather than adjusting their speed, the fish used a constant average speed, which was not linked to distance and was approximately constant over the path. This finding, however, allows rewriting of equation (4) in the form of

$$\begin{aligned} \Delta x &= \tau v_w \cos\beta \\ \Delta y &= \tau(v_w \sin\beta - v_F) \end{aligned} \quad , \tag{6}$$

in which τ is the actual time the fish needs to reach the impact point. This time is simply disposed with by one's considering the ratio D of the distances:

$$\frac{\Delta y}{\Delta x} = D = \frac{v_w \sin\beta - v_F}{v_w \cos\beta} \quad . \tag{7}$$

With the introduction of the speed ratio k = $v_F/v_W$, this relation can be rewritten as

$$\sin\beta - D\cos\beta = k \quad . \tag{8}$$

Solving for β yields our prediction (2) for the optimal initial aim that would lead the fish straight, at constant speed, to the fruit's later point of impact.

## Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, four figures, and two movies and can be found with this paper online at http://www.current-biology.com/supplemental/S0960-9822(08)01535-2.

## Acknowledgments

It is a pleasure to thank Luis Diego Gómez, Orlando Vargaz (La Selva), Otto von Helversen, Detlev Kelm, and Britta Lammers (Erlangen) for their help, the Organization for Tropical Studies (OTS, Duke) for preventing other people from feeding the La Selva fish, our referees for helpful comments, and the Deutsche Forschungsgemeinschaft for generous support.

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## Supplemental Data

## Supplemental Experimental Procedures

### *Analysis of speed relative to shore*

The average take-off speed was calculated from the change in coordinates of the fish's head between the frame that showed the fish's initial aim and the frame 40 ms later. For the more detailed analysis of post-start speed changes in Figure S2, head coordinates $H_1$-$H_4$ were taken every 20 ms, starting with the frame subsequent to that in which the fish's initial aim was measured. Three speed values $v_1$, $v_2$ and $v_3$ were computed from the respective shifts between $H_2$ and $H_1$, $H_3$ and $H_2$ and $H_4$ and $H_3$. For a crude estimate of the mean C-start linear acceleration we measured the take-off speed as the distance the head moved between the frame in which the initial aim was taken and the subsequent frame. Assuming a change from zero linear speed to take-off speed within 20 ms yielded our crude estimate of the fish's linear acceleration.

### *A consistent estimate of the prevailing effective flow*

The analysis of Figure 3 (see main article) shows that the initial aims were optimal not only for $k = 0.14$ but for a broader range $0.09 < k < 0.17$ of relative flow speed k. But is this the flow range that the fish actually did encounter in the stream? We approached this question in two ways: (1) First, we show that an estimate of $k = 0.14$ is consistent with our direct measurements of take-off speed relative to the shore and with the published speed (in body lengths per second) for comparable fish on comparably short journeys. (2) We also analyze the actual resulting movement of the fish after its start. This results from the vector addition of the fish's propulsion and the drift. If it is correct that the k value (or range of k values) – for which we found the initial aims to be optimal – coincides with the actual range of k values that acted on the fish in the stream, then extrapolating the resulting initial net movement should lead to a point close to the later impact point. In detail these two analyses were as follows:

(1) Because the average inclination of the resulting (relative-shore) speed vector $\vec{v}'$ with respect to water speed $\vec{v}_{flow}$ was 135° and because k is small we can simply approximate the absolute value of the fish's speed relative to the water $v$ as $v = v' + v_{flow}$. The relation

$$k = \frac{v_{flow}}{v} = \frac{v_{flow}}{v' + v_{flow}} \tag{1}$$

can thus be used to solve for

$$v_{flow} = v' \frac{k}{1-k} \tag{2}$$

.

Our estimate of k = 0.14 together with our measured average take-off speed relative to the shore of $v' = 2.4$ ms$^{-1}$ would thus yield an effective flow speed of $v_{flow} = 0.4$ ms$^{-1}$ and an average speed of the fish relative to the water of $v = 2.8$ ms$^{-1}$. Together with our direct measurements on the length (average 45 cm) of the fish in each response this would suggest that the fish moved at 6.2 body lengths per second, a value that is consistent with a survey of values found in similarly sized fish and with a required endurance of about 600 ms [S1].

(2) To check whether k = 0.14 is consistent with actual relative flow speed values we analyzed in detail the initial resulting movement of the fish's head for all those n = 34 starts in which the fish were undisturbed on their complete journey. We selected this particular dataset because assessing the resulting movement of the fish with reasonable accuracy requires sampling it over a long interval (80 ms) during which the fish could already have responded to the movement of other responding fish that moved in their way. Measuring the position of the head was most accurate and the head could be used as a marker because the orientation of the fish was stable within the 80 ms sampling interval. In each start we thus took head coordinates in 5 subsequent positions during the first 80 ms of take-off to determine the net initial movement direction by fitting a regression line to the five points. We then determined where this line would cross the y-axis (see Figure 3A), above or below the fruit's landing point. The average extrapolated resulting initial movement gave an intersection point that was slightly below the impact (10.5 ± 18 cm; mean ± SD, n = 34; regression lines, difference from zero $p < 0.01$, t-test) but certainly sufficiently close to it to say that the drift corrections actually did work for the prevailing flow.

*Generating the trajectories for en route drift-compensation in Figure 2A (see main article)*

A reference frame was used in which the flow is in the –y direction, the fish starts on the y-axis at a distance x = X from the goal point at (x = X, y = Y). Let the navigator be at position ($x_{n-1}$, $y_{n-1}$), aligned directly to the goal at an angle (to the x-axis) $\alpha_{n-1}$. We now assume that the navigator keeps this angle for a path length $v\Delta t$, which leads it to its next location at

$$\begin{aligned} x_n &= x_{n-1} + v\Delta t \cos(\alpha_{n-1}) \\ y_n &= y_{n-1} + v\Delta t(\sin(\alpha_{n-1}) - k) \end{aligned} \quad (3).$$

Here k is the speed of flow measured in units of the navigator's speed. At its new location the navigator corrects its angle so as to be again aligned to its goal point at (X,Y). This requires the new angle

$$\alpha_n = \arctan\left(\frac{Y - y_n}{X - x_n}\right) \quad (4),$$

that now is kept during the next step etc. Our iteration started with position (x = 0, y = 0) and (x = 0, y = 8). In both cases the goal was located at (x = 10, y = 10). In the examples given, the free path length vΔt was set equal to 1, i.e. 10% of the total x-distance.

## Supplemental Reference

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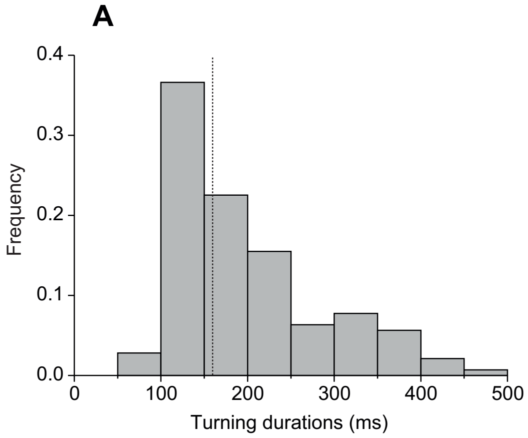
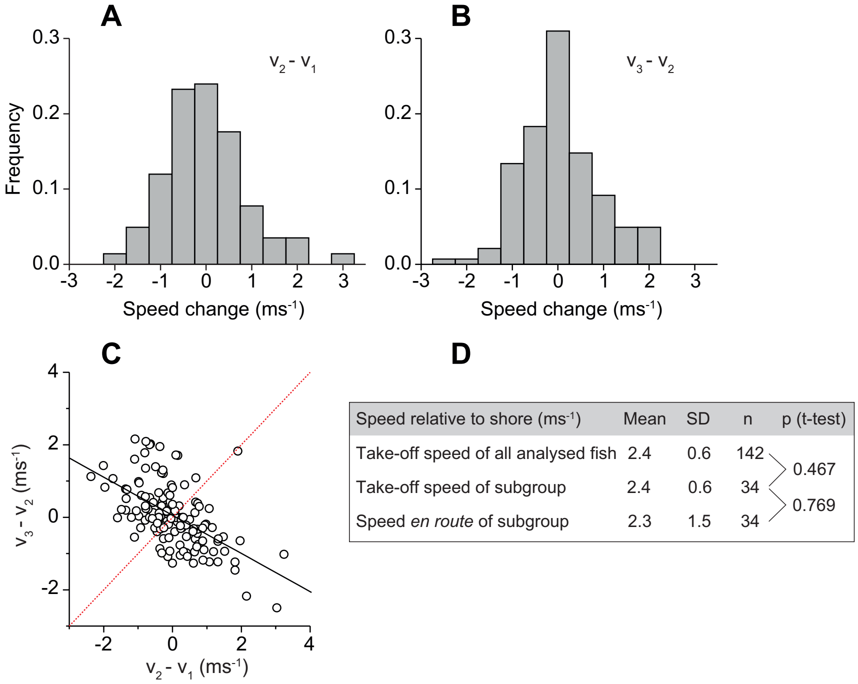


**B**

| | Mean | SD |
|---|---|---|
| $\dot{\phi}$ (°s$^{-1}$) | 260 | 110 |
| $a$ (ms$^{-2}$) | 115 | 50 |

Figure S1. Characteristics of the Fast Starts
(A) Distribution of turn duration in the n = 142 fast starts analyzed for their initial aim in Figure 3 (see main article). First bin 0-50 ms; dotted line shows median. (B) Table showing the average turning speed $\dot{\phi}$ (estimated for each response as the 'size of turn' / 'time needed to complete the turn') and the average estimated linear acceleration $a$ at the start of propulsion (n = 142).



| Speed relative to shore (ms-1) | Mean | SD | n | p (t-test) |
|---|---|---|---|---|
| Take-off speed of all analysed fish | 2.4 | 0.6 | 142 | 0.467 |
| Take-off speed of subgroup | 2.4 | 0.6 | 34 | 0.769 |
| Speed *en route* of subgroup | 2.3 | 1.5 | 34 | |

Figure S2. Following their Rapid Turns, the Fish Took Off with Constant Speed
To test whether the fish systematically changed their speed in the first 60 ms of their propulsion, we measured speed in three successive intervals, each of 20 ms duration. This yielded, for each of the $n = 142$ responses analyzed in Figure 3 (see main article), three speed estimates $v_1$ to $v_3$. The histograms show the distribution of the corresponding speed changes $v_2–v_1$ (A) and $v_3–v_2$ (B) in all responses. In both distributions the average was not significantly different from zero ($p = 0.97$ and 0.45, respectively, t-tests). (C) Correlation analysis to show that the scatter in the histograms (A, B) is not due to responses in which the fish had either been accelerating or decelerating. The occurrence of such events would predict a positive correlation (red line) between subsequent speed changes $v_3–v_2$ and $v_2–v_1$. In contrast, the actual correlation was negative ($r = –0.56$, $p < 0.0001$, black line), i.e. an apparent increase in speed tended to be followed by a decrease in speed and vice versa. (D) Speed of the fish relative to the shore. The take-off speed did not systematically increase with the distance of the responding fish to the fruit's later landing point ($r^2 = 0.06$, $n = 142$; regression not shown). The average take-off speed in a subgroup that brought the fish to the fruit's landing point, no later than 60 ms after the fruit's impact, was the same as that given above for all starts. In these paths, the take-off speed was not different from the fish's average speed *en route*.

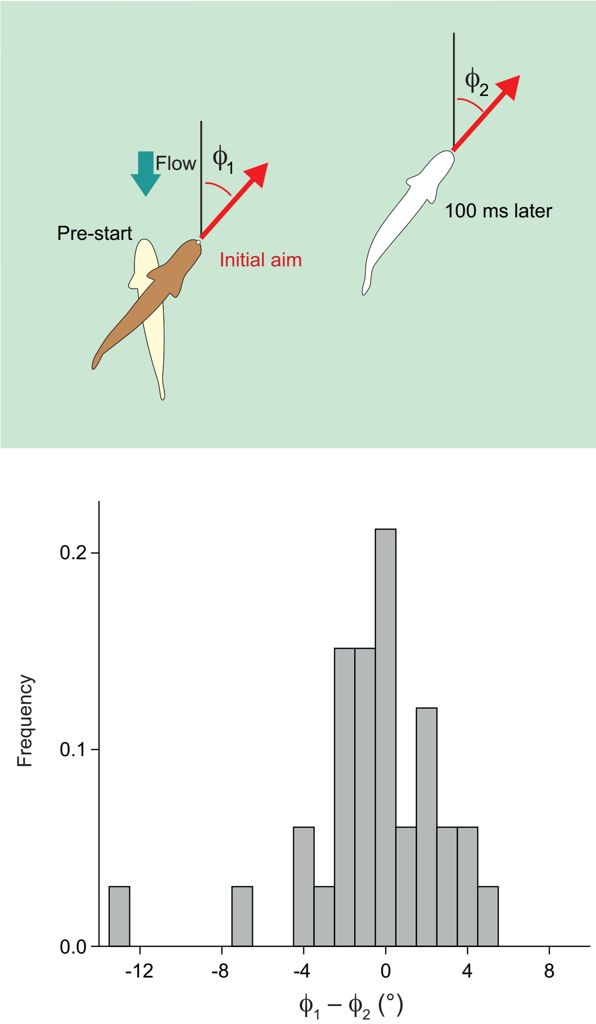


Figure S3. Following their Rapid Turns, the Fish Took Off with Constant Orientation

In 34 of the 142 responses of Figure 3 (see main article) the fish were not blocked by other fish on their later path towards the fruit's landing point. In 33 of these responses the orientation of the fish could be measured 100 ms after the initial aim was taken and still before the fruit landed in the stream. This allowed us to assay how constant the orientation was during the first 100 ms in 33 of the unblocked paths. In each path we measured the angle $\phi_1$ (initial aim versus the flow-axis) and $\phi_2$ (the aim 100 ms later versus flow-axis), as illustrated in the inset. The histograms show the distribution of the corresponding changes $\phi_2 - \phi_1$ in the orientation (bin width 1°, centre bin from –0.5° to +0.5°). The average (–0.57° ± 0.59°, mean ± SEM, n = 33) was not significantly different from zero ($p = 0.34$, t-test).

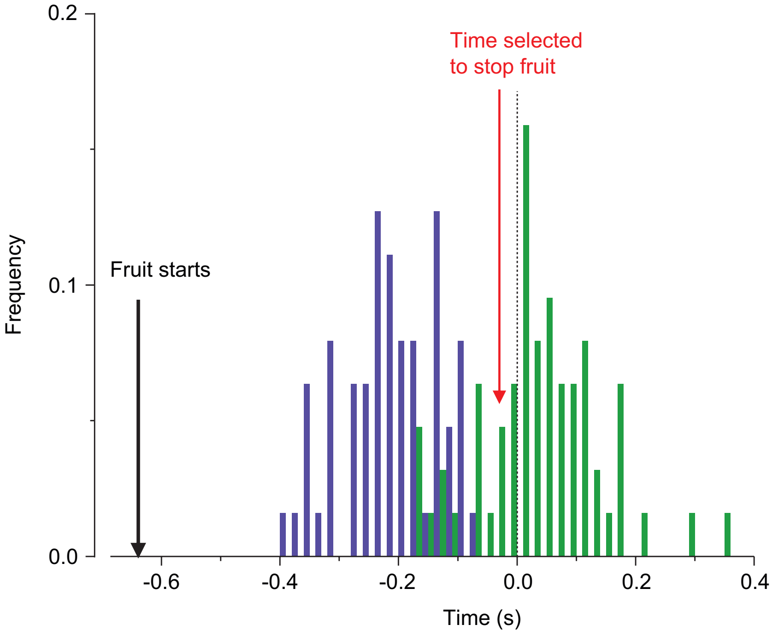


Figure S4. Timing Considerations for the Design of the Open-Loop Tests
Distribution of the response timing relative to the fruit's impact (at time 0.0 s, dashed line) when fruits fell freely from 2.0 m initial height. Bin width is 20 ms, and counts relating to the initiation (blue) and end (green) of the rapid turn are shown in the left and right half of each bin, respectively. First bin: –0.4 s to –0.38 s. The start of the fruit's motion (black arrow) is indicated. Stopping the fruit after 1.8 m of falling (at the time indicated by the red arrow) ensures that 100% of turns had been initiated whereas still 80% turns would not be finished. Therefore, the procedure ensures a large proportion of trials in which the stop occurs in the midst of the fish's rapid turn.

# Full adaptive plasticity in a vital circuit of the adult vertebrate brain

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Key words: plasticity/stability tradeoff, adult plasticity, functional stability, adult brain

**The need to maintain stable function is thought to limit adaptive plasticity in the adult brain but the limits of such a stability-plasticity trade-off are presently unknown[1,2,3,4]. Here we show that a vital hindbrain circuit[5,6,7], despite its simplicity and importance of maintained functionality, is capable of full adaptive plasticity. Suitable incremental training[8] of adult zebrafish showed that the circuit not only learns to adaptively use novel sensory information – which initially it could not – but that it can *de novo* extract and represent the law that guides success. Using a behavioral approach we demonstrate that the network had learned to represent the underlying law of physics rather than operating on a set of stored memory templates. Hence, even vital circuits of the adult brain can maintain full plasticity without compromising their other important functions. Our findings pave the way for unraveling the conditions that enable full plasticity – while maintaining functionality – in a vital circuit of the adult vertebrate brain.**

Adaptive plasticity plays a crucial role in the brain but the nature and degree of experience-dependent plasticity differs between mature and juvenile animals[3,9,10]. To understand neural plasticity in the mature animal it is essential to elucidate its fundamental limitations. One major concept is that some sort of trade-off exists between plasticity and stability[1–4] so that adult plasticity needs to be restricted to ensure stable functionality. While the adult mammalian cerebral cortex and the avian forebrain are widely acknowledged for their high degree of adult plasticity[11,12,13] other areas with more basic vital functions would have to be shielded against plasticity so that their functionality would not be compromised during phases of plastic readjustments. In this study we use behavioral techniques to probe the degree of flexibility in a vital behavior of an adult vertebrate. Fish and tadpoles use rapid Mauthner-cell driven C-starts to escape from life-threatening situations[5,6,14], but can use such starts also in the context of feeding[15,16,17]. This vital behavior is mediated by reticulospinal networks whose major cells are interconnected with many sensory and brain areas using a wide range of synaptic systems[6,7]. In adult zebrafish, C-starts can not only be driven by predators but also by water waves that emerge from food that has fallen onto the surface (Fig. 1a), a large part of the diet of zebrafish in the wild[18,19].

Given the importance of fully maintained functionality of the circuits that drive these responses at reflexlike speed, we asked to which extent they would be capable of adaptive plasticity. Specifically, we explored if adult fish could be trained to elicit adaptive responses based on visual instead of mechanosensory information and on the acquisition of a novel visuomotor transformation. Training was incremental[8] and started with 10 weeks of 2500 trials in which the only food available was aerial and was sent on ballistic tracks from 35 cm initial height. Initially tracks were always in the same direction but horizontal speed was varied randomly from test to test (in the range from 0.19 to 1.34 $\mathrm{ms}^{-1}$). The experimental design was such that the fish did not know when and at which speed the food was moving so that the visual information the zebrafish could potentially use was under full experimental control. Surprisingly, after this initial training phase the fish regularly elicited visually guided fast-starts so that they were already on their way right before their food landed (Fig. 1a). In a subsequent set of 1000 trials we made the task considerably harder by varying both speed and direction of the initial motion randomly and independently from each other from one trial to the next. As we will show below, the fish learned to handle arbitrary and novel combinations. Moreover, monitoring the key

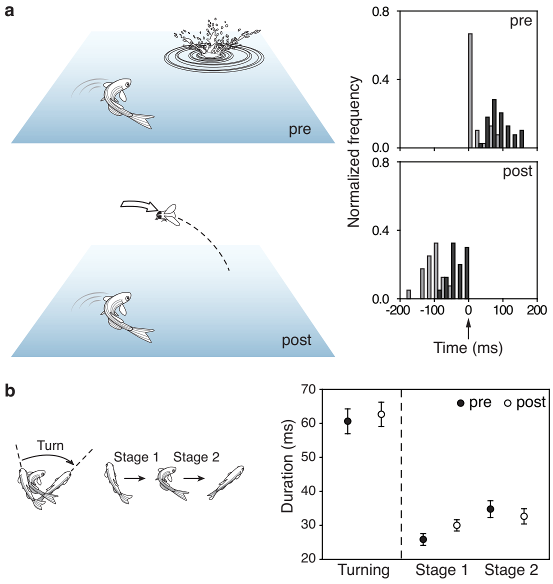

**Figure 1. A vital adult circuit learns a novel sensomotor transformation. a**, Zebrafish can launch fast-starts upon the splashing impact of food. When all available food falls on ballistic paths towards the water surface, zebrafish learn to use visual cues to trigger directed starts towards the later impact point. Histograms show onset of fast-start (grey, first half of each bin) and of actual take-off (black, second half of bin) in untrained fish (top, 'pre'; $N = 39$) and after extensive training (bottom, 'post'; $N = 40$). Bin width 20 ms. **b**, Evidence that same motor pattern is elicited after training. No significant difference in total duration ($P = 0.64$, Mann-Whitney U-test), duration of so-called stage 1 ($P = 0.08$, U-test) and stage 2 (subsequent straightening phase; $P = 0.48$, U-test) of the initial and post-training starts. Same starts in **a** and **b**.

characteristics of the post-training starts directly showed that the same type of motor response was produced as before training had started (Fig. 1b and Extended Data Figure 1) – but now elicited and guided by different information.

Figure 2 shows that already the second stage of training had led to remarkable performance, allowing the fish to accurately turn to a point from which no cues emanate and that they could only infer from an analysis of visual motion. For any combination of initial speed and direction the fish were already turned and on their way to the later point of catch while the food was still in the air and the aim was taken so well that no later corrections were needed (Extended Data Fig. 2). Demonstrably, their aims were not related to any point on the actual trajectory (quantified by minimum error e' made to a point on the pre-start trajectory) but truly to a point from which no cues emanated when the start was initiated. The visually driven post-training starts were, furthermore, equally well-aimed over the full angular range (Fig. 2b). Moreover, throughout the learning phase the fish not only readily responded to threatening stimuli but also were able to elicit mechanosensory driven C-starts.

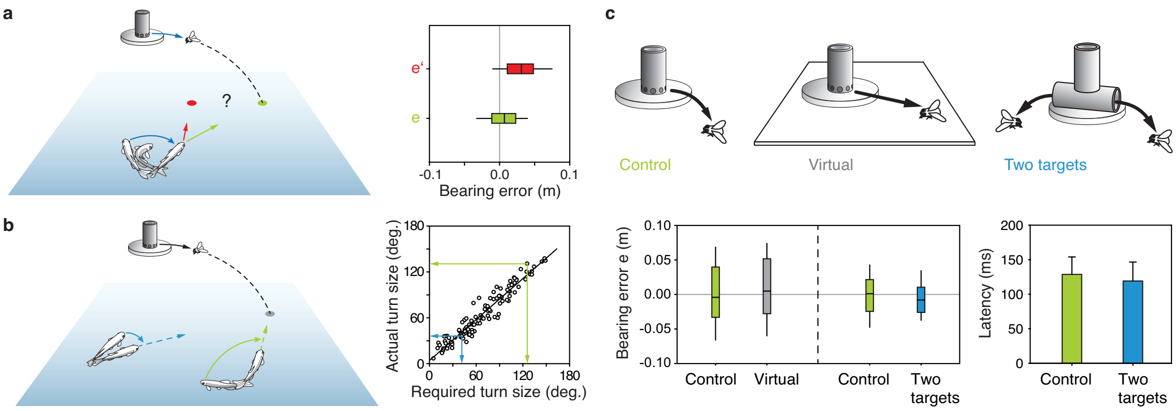

**Figure 2. Training provides the circuit with high-resolution information on aerial movement.** **a**, Trained zebrafish turned right to the later point of impact (green) but not to the projected actual position (red) of the falling food. Their turns minimized the error e (green, difference to zero, $P = 0.07$, t-test) with respect to the later landing point but not the error made to the actual post-turn target position (error e', red, difference to zero $P < 0.001$, t-test, $N = 119$ starts). **b**, After training, zebrafish can produce correct visually driven turns over the full angular range. Sketch illustrates two adaptive starts (blue, green) made to same initial motion of food. Plot shows how training has led to a tight relation between actual and required turns ($R^2 = 0.92$, $N = 110$, $P < 0.001$). **c**, The response is fed with high-resolution data on horizontal movement. 'Virtual': Among ballistic falling patterns (normal, $N = 451$) a few tests (virtual, $N = 47$) were occassionally interspersed, in which food slid on a glass plate. This fooled the fish to turn to the respective virtual impact point at which food with the given set of initial motion parameters would land (Difference 'Virtual' / 'Control' $P = 0.39$, t-test). 'Two targets': When confronted with conflicting sets of initial motion values the fish chose one set and responded equally accurate as in controls (two targets $N = 84$, control $N = 110$, $P = 0.56$, t-test). This added difficulty did not increase (but slightly decrease $P = 0.01$, t-test) latency. Boxplots: Boundaries (whiskers) show 25th/75th (10th/90th) percentiles, lines mark median.

Which visual information had the fish learned to extract to guide their early starts? In a series of experiments to address this issue the fish were supplied with information on horizontal speed and direction but no vertical movement was allowed. Should the visual starts be based on a true representation of the three-dimensional trajectory, the fish should either not respond at all (because nothing comes down) or turn to a very distant landing point. In the experiments, however, the fish responded just as with the vertical component present and turned to the respective 'virtual' impact point. This point could be predicted on assuming that the fish drove their response only based on the initial horizontal speed and direction (Fig. 2c; control *versus* virtual).

We next probed the accuracy at which the trained fish were able to provide their choice circuitry with information about target speed and direction. In these experiments two identical prey items took off simultaneously in opposite directions. Rather than responding to the average of the motion signals the fish selectively turned to the landing point of only one of the two items, completely neglecting the motion cues of the other item. The selection was not made at random but based on which of the two landing points required the lesser turn (Extended Data Figure 3). Remarkably, the choice did not reduce accuracy nor did it increase the latency of the response (Fig. 2c control *versus* two targets). The fish were thus not averaging movement across larger areas but pinpointing speed and position.

To discover how the visuomotor transformation was programmed into the decision circuit we probed the fish with arbitrary initial value combinations that they had not encountered during training. One possibility was that the fish had stored a number of 'memory templates', i.e. which turn was rewarded for a given combination of initial speed and direction of the target and for a given initial position and orientation of the responding fish. In any acute situation the fish would then interpolate within this stored set of templates. Because all prior training occurred at one initial target height level, template storage can be tested by challenging the fish with non-training height levels. At such levels a predictable error should occur that would readily be measurable. In the experiments to test this prediction, the majority of food items still fell from the training height, although speed and direction were randomly changed from one trial to the next. The novel height levels were only occasionally used with motion selected randomly (see supplementary materials). Surprisingly, the fish readily handled the novel situations and the accuracy with which they took aim in the novel situation was not different from that in the familiar training situation (Fig. 3). The visuomotor transformation had thus not been made on the basis of templates but on the basis of the general rule that connected initial position, speed and direction of target motion to the required turn.

Our findings raise the intriguing possibility that the fundamental balance between stability and plasticity, a major limitation of adult neural plasticity[1,2,3,4], can be outmaneuvered, surprisingly in an apparently simple hindbrain circuit of a fixed number of identified cells. We have studied a vital life-saving behavior whose function must by no means be compromised and had therefore expected to find clear limitations in its plasticity. However, not only is the adult circuit fully capable of feeding in rather unusual high-resolution information on aerial ballistic motion, accurately judged despite the position dependence of refraction at the water-air interface[20], but can even represent the general characteristics of ballistic motion, thus acquiring a novel complex visuomotor transformation without any signs of its other functions being compromised. Given the outstanding accessibility of the circuitry in zebrafish at one hand[21,22,23,24] and the emerging fundamental insight into the mechanisms that balance the plasticity/stability trade-off in the adult brain[4,25,26,27] we suppose that our findings will pave the way to discover the mechanisms that allow such an unusual way of outmaneuvering the fundamental limitations of adult plasticity.

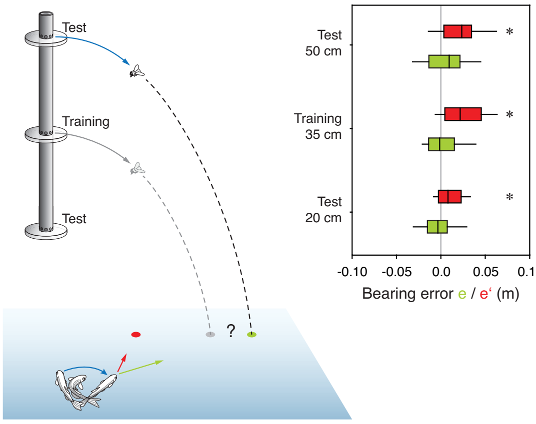

**Figure 3. The visuomotor transformation operates on a stored concept rather than on memory templates.** After exclusive training at only one height level (Training; 35 cm) fish were probed in tests with novel height levels (Test; 20 cm or 50 cm; initial movement speed and direction selected at random). These tests were interspersed among presentations at the training height. Should the fish decide based on memory templates (which exists only for the training height) they should start towards an erroneous position (grey). Box plots (conventions as in Fig. 2) show that the fish turned precisely also in the novel situation (green; difference from zero in all cases $P > 0.05$, t-test; difference between heights $P = 0.48$, one-way Anova) and did not switch to an approximate non-predictive strategy (red; asterisks: significant difference e' to e, $P < 0.001$ in all cases, paired t-tests; $N_{20cm} = 34$, $N_{35cm} = 33$, $N_{50cm} = 25$).

## Methods Summary

Adult zebrafish that produced C-starts in response to the splashing impact of food and in response to threatening stimuli were subjected to incremental training in which they eventually had to predict the future landing point of food that fell from a certain height with its initial horizontal speed and direction not known a priori to the fish and changed randomly from one trial to the next. C-starts were recorded from above and the accuracy of the initial direction (as soon as the C-start was finished) with respect to the later impact point (or alternatives as described) was assayed after suitably correcting for distortions in perspective. To analyze the nature of the novel representation acquired by the trained fish we performed tasks in which the natural movement pattern was changed to produce predictable errors and in which prey could be started from novel non-training height levels.

**Online Content** Additional Methods and Extended Data display items are available in the online version of the paper.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature

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**Author Contributions** S.S. conceived the study. P.K., M.V., R.B. and S.A. performed experiments. All authors analyzed data. P.K. and R.B. wrote algorithms. P.K. and S.S. wrote the paper.

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## Methods

***Animals.*** A group of thirteen adult zebrafish, *Danio rerio* Hamilton 1822 (standard length 4 cm) was used. Fish were kept and all experiments conducted in a large tank (1.2 m × 0.5 m × 0.5 m) filled to a height of 10 cm with fresh water (temperature 25°C). Animals were subjected to a 12 h:12 h light regime and all experiments started not earlier than 3 hours after light onset to ensure the fish were fully light adapted.

***Setups and protocols.***

*(1) Training and basic tests.* Food was blown from a transparent circular platform (acrylic glass, 30 mm in diameter), mounted 35 cm above the water surface in the center of the tank. The center of the platform held a flexible tube (10 mm in diameter) with eight equally spaced air-valves (3 mm diameter each) out of which an air current could be directed at a prey item (dead fly without head and wings, *Calliphora spec*) placed on the 10 mm rim. Displaced by the air current the fly left the platform and fell ballistically. The activation of the air current was not visible to the fish. To provide the responding fish with an immediately available reward we prepared the flies by coating them with fine particles of ordinary fish food (Tetra Min). In the initial training (2500 trials in 10 weeks) only one of the eight air-valves was used, however speed was varied from 0.19 to 1.34 m $s^{-1}$. In the subsequent training (1000 trials) and later testing phase each of the eight air-valves was used in random order, so that prey take-off could be varied over the full range of angles (see Fig. 2b) in addition to the variation in initial speed. This training at the height level of 35 cm was kept throughout the full duration of the study (i.e. between the different types of experiments described below). Michelson contrast between the falling fly (reflected light 34.5 cd $m^{-2}$) and its background (reflected light 190.4 cd $m^{-2}$) was 0.69 in all experiments in which timing information was determined.

*(2) Virtual impact point.* To test which characteristics of the aerial trajectory the trained fish were using to guide their starts we fooled the fish with flies that actually did not come down at all. This was achieved by placing a horizontal glass screen underneath the platform described above (see also inset of Fig. 2c ‘Virtual’) so that the fly slid horizontally on the glass plate without ever coming down. These tests were interspersed at a low average rate of 1 in 10 presentations among tests with ballistically falling flies.

*(3) Transfer tests.* Three of the standard platforms were mounted on a vertical column (see Fig. 3) so that one fly at a time could be catapulted either from 20 cm, 35 cm and 50 cm

above the water surface. Prior to these tests the fish had exclusively been trained at motion starting from 35 cm height and the fish received a further set of 1000 trials at 35 cm starting height immediately before the transfer testing began. Two measures were taken to ensure that the fish could not learn during the transfer tests: (1) Testing occurred in two blocks with 500 trials at only 35 cm initial height between them. (2) In the test blocks the rate at which objects started from novel height level was chosen low (one of ten tests).

*(4) Two-target experiments.* To simultaneously release two flies in opposing directions, we pivot-mounted an inverted T-tube (internal diameter 8 mm) onto the usual platform as described for the initial testing phase (see inset Fig. 2c ‘Two targets’). Before each experiment the shaft (length 20 mm) was rotated to a randomly chosen direction. It was ensured that the fish were not able to see the orientation of the T-tube. A stream of air into the inverted T-tube started the motion of the two flies. To ensure approximately matched speed levels the two T-ends were fitted with similarly sized flies. It was checked that the two flies left simultaneously (with 4 ms accuracy) the two shafts in opposing directions and only cases with confirmed equal appearance were analyzed and compared to randomly interspersed tests in which only one fly was blown out of the T-tube (at the absolute ratio of 1:1). Group size was 9 fish in these experiments.

***Recording.*** All recording was made from above with the camera monitoring a sufficiently large area of approximately 0.5 m × 0.5 m at a right angle. For better contrast the tank was diffusely illuminated from below. In the initial experiments (Fig. 2a) and the tests at 3 different height levels (Fig. 3) we recorded responses at 50 frames per second, using a Sony Digital 8 DCR-TRV270E, in all other experiments we used digital highspeed video. The kinematics of the fast-starts (Fig. 1 and Extended Data Fig. 1) were analyzed at 250 frames per second (Photron Fastcam MC2). In the experiments of Fig. 2b–c, Extended Data Fig. 2 and Extended Data Fig. 3 frame rate was 500 per second (HotShot 1280M, NAC Image Technology).

***Data analysis.*** All processing of the video material was done using NIH Image J and custom written software.

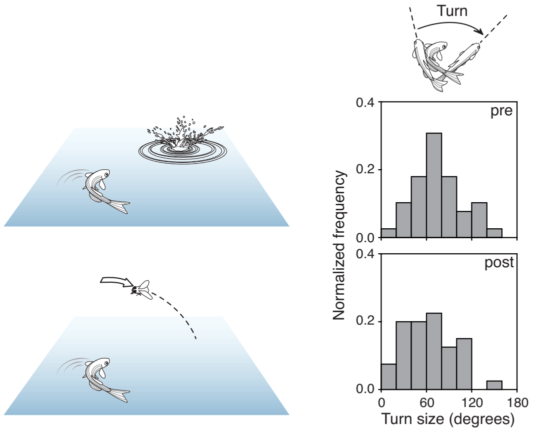
*(1) Timing*. The measurements we considered were the latency, the C-start kinematics and the accuracy of the fish’s rapid predictive turns. Latency was derived from the number of frames between the onset of prey movement and the initiation of the C-Start. Duration of the C-start and its two stages were similarly taken. Stage 1 was defined as the time the fish

needed from initiating the turn until the fish's body was maximally bent, followed by stage 2, from maximum bend till the end of the turn leading to the subsequent take-off phase.

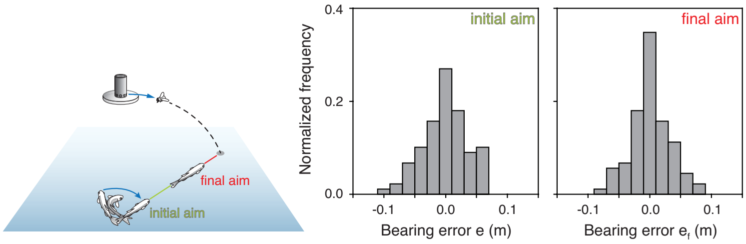
*(2) Distortion in perspective.* Because the camera viewed target and fish at different distances a distortion correction was required in all metric measurements such as the assessment of the initial aim. At each actual height of the falling prey, its position was projected to the water surface, the plane in which the zebrafish acted. Thereby we took the known distortion in perspective for that height into account. Because the dislodged prey followed the laws of ballistic falling without air friction, the actual height of the falling prey could simply be calculated from the time of falling.

*(3) Accuracy.* Accuracy of the fish's initial aim was assessed as follows: As soon as the fish was straight at the end of stage 2 of its C-start and prior to its actual take-off the orientation of the fish was taken to derive the bearing error e with respect to the later point of prey impact (P) or the error e' made with respect to the horizontally projected position of the prey at the end of stage 2 of the fish's response (P'). By convention, e (e′) was defined as negative if the distance S–I < S–P (P′), where S is the initial target position and I is the point of intersection between the path of the fish and the projected path of the fly. In the glass plate experiments the *virtual* point of impact, at which the prey would have hit the water if it had been allowed to fall, could be calculated from simple ballistics of the prey's initial movement (height, direction and speed). In the two flies experiments we analyzed the error of the fish's initial aim (at the end of its C-turn) with respect to the later impact of the chosen fly. To ensure that we assessed accuracy and latency only when no other than cues from the falling motion of prey were available we took data only from the first fish that responded and restricted the analysis to responses in which the prey was still falling when the fish started to take off (so that mechanosensory input from the splashing prey was irrelevant). The path to the later point of impact had to be free of obstacles (such as nearby conspecifics) as obstacles might affect the initial aim. To exclude responses in which the fish could simply continue along their initial direction, a minimal turning angle of 10° was required.

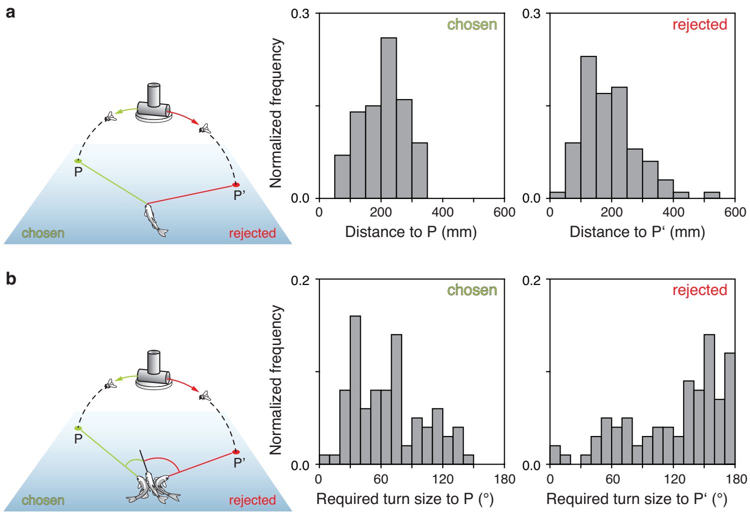
*(4) Statistics.* All statistical tests were performed two-tailed with an alpha level of 5% with Sigma Plot (version 11.0, Systat Software Inc. 2008). Normality of data was checked using Shapiro-Wilk test.



**Extended Data Figure 1. Comparable size of responses in Fig. 1.** Histograms show distribution of turn size in the pre- and posttraining starts whose kinematics were analyzed in Fig. 1. Pre-training $N = 39$ turns, post-training $N = 40$ turns. 20° bin width. No significant difference in turn size ($P = 0.10$, t-test).



**Extended Data Figure 2. Initial aim is accurate and requires no net correction.** After training, the visually driven fast-start were so well aligned that the distribution of the error in initial aim (green) does not differ from the distribution of the error immediately (5-10 frames, taken when fish was straight) before the point of catch (red). No significant difference between distributions ($P = 0.52$, paired t-test; $N = 89$; bin width 0.02 m).



**Extended Data Figure 3. Zebrafish have access to high-accuracy data to select among conflicting stimulus configurations.** Trained zebrafish simultaneously faced two targets that left the platform in opposing direction. In this situation they selectively take the motion of only one of the targets into account. Target choice is not random. **a**, Choice was not based on distance between responding fish and later landing point (bin width 50 mm, Wilcoxon signed rank test: $N = 84$, $P = 0.258$). **b**, Choice is predicted by the size of the required turn (bin width 10°, Wilcoxon signed rank test: $N = 84$, $P < 0.001$).

**Extended Data Table 1.** Means, s.e.m. (standard error of the mean), median, 25% and 75% percentiles for all results reported in the figures.

| | | | | *N* | mean | s.e.m. | median | 25% percentile | 75% percentile |
|---|---|---|---|---|---|---|---|---|---|
| **Figure 1** | a | Start of turn (ms) | pre | 39 | 23.90 | 4.35 | 12.00 | 4.00 | 32.00 |
| | | | post | 40 | -102.00 | 4.24 | -100.00 | -120.00 | -90.00 |
| | | End of turn (ms) | pre | 39 | 84.51 | 5.05 | 80.00 | 60.00 | 112.00 |
| | | | post | 40 | -39.40 | 4.02 | -42.00 | -58.00 | -58.00 |
| | b | Turning (ms) | pre | 39 | 60.62 | 3.65 | 56.00 | 48.00 | 75.00 |
| | | | post | 40 | 64.44 | 4.96 | 60.00 | 47.00 | 73.00 |
| | | Stage 1 (ms) | pre | 39 | 25.85 | 1.74 | 24.00 | 16.00 | 35.00 |
| | | | post | 40 | 30.00 | 1.67 | 28.00 | 22.00 | 39.00 |
| | | Stage 2 (ms) | pre | 39 | 34.77 | 2.47 | 32.00 | 25.00 | 40.00 |
| | | | post | 40 | 32.65 | 2.25 | 30.00 | 24.00 | 40.00 |
| **Figure 2** | a | Bearing error (m) | e | 119 | 0.008 | 0.003 | 0.007 | -0.011 | 0.023 |
| | | | e’ | 119 | 0.032 | 0.003 | 0.031 | 0.011 | 0.048 |
| | c | Bearing error (m) | Control (Virtual) | 451 | 0.002 | 0.003 | -0.004 | -0.033 | 0.040 |
| | | | Virtual | 47 | 0.009 | 0.007 | 0.005 | -0.026 | 0.052 |
| | | | Control (Two targets) | 110 | -0.002 | 0.003 | 0.001 | -0.024 | 0.022 |
| | | | Two targets | 84 | -0.004 | 0.003 | -0.008 | -0.026 | 0.010 |
| | | Latency (ms) | Control (Two targets) | 110 | 128.66 | 2.41 | 130.00 | 114.00 | 144.00 |
| | | | Two targets | 84 | 119.07 | 2.99 | 122.00 | 104.00 | 135.00 |
| **Figure 3** | | Bearing error e (m) | 50 cm | 25 | 0.006 | 0.006 | 0.009 | -0.013 | 0.021 |
| | | | 35 cm | 33 | 0.000 | 0.005 | -0.001 | -0.014 | 0.015 |
| | | | 20 cm | 34 | -0.003 | 0.005 | -0.003 | -0.014 | 0.007 |
| | | Bearing error e’ (m) | 50 cm | 25 | 0.021 | 0.005 | 0.024 | 0.005 | 0.035 |
| | | | 35 cm | 33 | 0.023 | 0.005 | 0.022 | 0.005 | 0.044 |
| | | | 20 cm | 34 | 0.010 | 0.004 | 0.008 | -0.002 | 0.023 |
| **Ext. Data Figure 1** | | Turn size (degrees) | pre | 39 | 76.30 | 5.15 | 70.56 | 55.24 | 95.77 |
| | | | post | 40 | 64.44 | 4.96 | 63.62 | 39.50 | 84.95 |
| **Ext. Data Figure 2** | | Bearing error (m) | e | 89 | -0.001 | 0.004 | 0.002 | -0.023 | 0.022 |
| | | | $e_f$ | 89 | 0.001 | 0.003 | 0.000 | -0.017 | 0.019 |
| **Ext. Data Figure 3** | a | Distance (mm) | to P | 84 | 207.35 | 7.66 | 212.22 | 154.51 | 257.87 |
| | | | to P’ | 84 | 195.59 | 9.75 | 176.44 | 131.12 | 242.78 |
| | b | Required turn size (degrees) | to P | 84 | 68.46 | 3.71 | 66.29 | 38.93 | 92.42 |
| | | | to P’ | 84 | 46.94 | 5.12 | 137.01 | 81.04 | 158.18 |

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